

# Toxicology News

June 2003

An AACC/CAP Educational Newsletter for Toxicology Laboratories

## Methadone: An Old Drug Achieves New Notoriety

By Ruth E. Winecker

**A**n increase in reports of methadone toxicity from hospital emergency rooms and medico-legal death investigations has generated renewed interest in this narcotic analgesic. Methadone is a synthetic opioid agonist first developed in response to morphine shortages during World War II. Best known for its use as a treatment for heroin addiction, recently it has been used as a less expensive alternative to sustained release opiate preparations, including morphine (MS-Contin), oxycodone (Oxycontin), and fentanyl (Duragesic), in chronic pain management and palliative cancer care.

Table 1 compares the features of methadone and selected sustained-release opioids. About as potent as morphine, methadone hydrochloride (Dolophine, Methadose) is available as a racemic mixture of isomers in a variety of dosage formulations. Oral products include 5- and 10-mg tablets; a 40-mg diskette/wafer; and solutions of 1, 2, and 10 mg/mL. A 10-mg/mL solution for parenteral injection and a 50g/100g powder are also available.

### Chemistry and pharmacology

**Chemistry:** Described chemically as 6-dimethylamino-4,4-diphenyl-3-heptanone, methadone has a chiral center on the sixth carbon and is usually found

in a racemic mixture of (R)- and (S)-enantiometric forms. Figure 1 illustrates the racemic forms.

**Dosages:** The size of the prescribed dose of methadone depends on the purpose of its use and the tolerance of the patient to opiates. Generally, methadone maintenance patients can ultimately take 60–100 mg/day. However, many oversight agencies recommend a maximum of 15–30 mg/day for the first three days of treatment with a gradual titration to a clinically effective dose due to a high risk of accidental overdose during the first 14 days of treatment (1, 2). Chronic pain and cancer patients who are new to opioid therapy or are stepping up from a weaker opioid usually begin with 7.5–30 mg/day. Regardless of the purpose of methadone treatment, dosages can eventually exceed 200 mg/day (2).

**Absorption and drug levels:** Absorption after oral ingestion is efficient and timely with an average oral bioavailability of 80% (range 67–95%). Single and chronic doses administered orally exhibit peak plasma concentrations 2.5 hours for solutions and 4 hours for tablets post-ingestion. Peak plasma levels range from 75 ng/mL after a single 15-mg dose to an average of 830 ng/mL for 100–200 mg/day chronic dosing (3, 4). As expected, parenteral injections result in higher plasma levels (96-ng/mL for a 10-mg injection in the deltoid region) and faster time to peak (34 minutes) than oral ingestion.

**Distribution:** Being lipophilic and basic, methadone distributes widely to tissues and is highly protein-bound. Volume of distribution (Vd) estimates vary and appear to depend on the test population, with naive volunteers, opiate addicts, and chronic pain patients ranging from 3.8–5 L/kg, 4.2–9.2 L/kg, and 1.7–5.3 L/kg, respectively

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**Table 1. Comparison of Methadone, MS-Contin, OxyContin, and Duragesic**

Drug	Peak (h)	t <sub>1/2</sub> (h)	Oral bio-availability	Equivalent dose (mg)	Vd (L/Kg)	Cost/month (U.S. dollars)*
Methadone	2.5–4	15–55	67–95%	20	1.5–9.2	10
MS-Contin	3	2–4	40%	60	4	240
OxyContin	3	4.5	60–87%	30	2.6	150
Duragesic	34–38	3–12	n/a	n/a	3–8	240

\*Source: Clinical Pharmacology 2000 Monographs, <http://cpip.gsm.com/>. Accessed April 3, 2003.

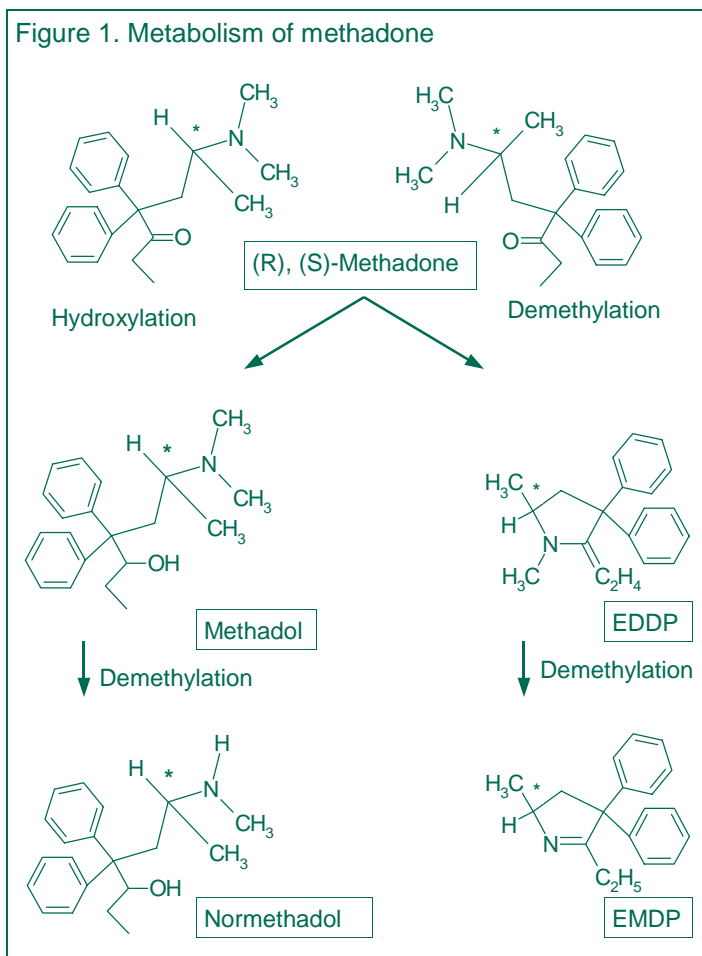
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## Methadone

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Figure 1. Metabolism of methadone



(3, 4). In plasma, methadone is predominantly bound to  $\alpha_1$ -acid glycoprotein (AAG) with a free fraction averaging 0.15. Interestingly, the free fraction appears to be considerably higher in naive healthy volunteers compared with cancer patients or methadone maintenance patients. This may be due, in part, to increases in AAG levels during stress conditions and may have a role in the toxicity seen in the casual user (4).

**Metabolism:** Methadone is biotransformed predominantly by hepatic routes with a minor fraction metabolized during absorption by isoenzymes (CYP3A4) in the intestinal wall (2). In the liver, mono- and di-N-demethylation result in the production of unstable metabolites, which rapidly undergo cyclization to form 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and to a lesser extent 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP), respectively. The majority of demethylation is carried out by the hepatic CYP3A4 isoenzyme with minor contributions from CYP2C19, CYP2D6, and

CYP2C9. Methadone, EDDP, and EMDP undergo hydroxylation in the para position on one of the two-phenyl moieties and are further conjugated with glucuronide. Methadol is formed via hydroxylation of the keto group on the third carbon and is further metabolized to normethadol via the same demethylation processes described above. Although produced in minor concentrations, both methadol and normethadol exhibit pharmacological activity similar to that of methadone (3, 4). Figure 1 illustrates the biotransformation of methadone.

**Elimination and clearance:** The major elimination pathways for methadone and its metabolites include fecal and renal. Renal excretion rates are highly dependent on urinary pH and can range as low as 4% when urine pH is above 6.0 to 30% for acidic urine (pH <6) in healthy volunteers. The half-life of methadone has been estimated to be 15–55 hours. Clearance of methadone is complicated by induction of the CYP3A4 isoenzyme during multiple dosing and may contribute to the high variation in half-life. According to at least one study, up-regulation of CYP3A4 resulted in a 3.5-fold increase in clearance in chronic users (4).

These unpredictable changes in the rate of metabolism and clearance can make interpretation of urine drug levels difficult. In a single-dose study of healthy individuals, methadone and EDDP accounted for 5% of a 5-mg oral dose in a 24-hour urine collection. In contrast, 24-hour urine studies of methadone maintenance subjects indicate methadone and EDDP may account for 5–50% and 3–25% of a dose, respectively (4). An additional consideration is the high potential for drug–drug interactions due to concomitant administration of drugs that either induce or inhibit CYP3A4 (1, 4). Table 2 lists a number of reported drug interactions.

**Stereochemistry issues:** Although most formulations of methadone are a racemic mixture of (R)- and (S)-isomers, studies comparing the efficacy of

**Table 2. Notable drug interactions**

Agents causing decreased methadone plasma levels:

Barbiturates, carbamazepine, chronic ethanol, cocaine, phenytoin, nevirapine, rifampin, ritonavir, Vitamin C

Agents causing increased methadone plasma levels:

Acute ethanol, benzodiazepines, cimetidine, diazepam, ketoconazole, selective serotonin re-uptake inhibitors, tricyclics, zidovudine

Drugs affected by concomitant methadone use:

Nifedipine: Decreased metabolism of nifedipine  
Zidovudine: Inhibited zidovudine glucuronidation

racemic dosing to (R)-methadone indicate that even when delivered at twice the (R)-methadone dose, patients on the racemic dose exhibited more dissatisfaction with their maintenance treatment and usually demanded a dose increase (1). This finding is consistent with studies concluding that (R)-methadone has a much greater affinity for opioid receptors compared with (S)-methadone and that (R)-methadone is responsible for the vast majority of the total analgesic effect. These stereo-selective effects are also seen in the pharmacokinetics of methadone; studies have shown differences in the volume of distribution and clearance of (R)- and (S)-methadone. These differences, however, appear to be less a function of differences in metabolism than in protein binding and genetic expression of CYP isoforms (1, 4).

### Medicinal use

Methadone has been used in the United States since the 1960s as a medical intervention for heroin abuse. Approximately 20% of the estimated 1 million U.S. heroin addicts are current participants in methadone maintenance treatment (MMT) programs.

Prior to 1999, these programs were regulated by comprehensive protocols established by the Food and Drug Administration. These regulations covered everything from operating practices to restrictions on the drug's use and availability.

To increase participation in MMT programs, the Department of Health and Human Services announced sweeping guideline changes in July 1999 that allowed for greater flexibility and clinical discretion by participating physicians. Under the new guidelines, MMT programs would establish quality assurance guidelines for a clinical standard of care and would then be accredited by a national or state accrediting body (5). On the downside, these guidelines may have unwittingly allowed for more diversion to illegal use by relaxing the regulations on the amount of methadone a patient may take home (6, 7).

Proponents of MMT programs state that methadone reduces or eliminates heroin abuse by blocking the euphoric effect of concomitant heroin use and suppressing the abstinence syndrome (withdrawal). This in turn leads to a reduction in the transmission of hepatitis and HIV/AIDS, a decrease in narcotic-related crimes, and an increased likelihood that the methadone maintenance patient will lead a more productive life. Studies indicate that among MMT participants, weekly heroin use decreased 69%, criminal activity decreased 52%, and full-time employment increased 24% (1, 5). Large daily doses (80–120 mg) of methadone and high participation scores (defined as high percentage of drug-free urine tests and attendance at most counseling sessions) are indicative of a

successful outcome for a patient participating in an MMT program (1).

Methadone has also been used to treat cancer and chronic pain. Strong narcotic analgesics are considered the best prescription choice for cancer patients with moderate to severe pain. This is not inconsequential, as 80% of cancer patients will experience moderate to severe pain during their illness and 20% of them will be either unresponsive to or intolerant of traditional morphine-like opiates. Methadone can be used as an alternative opioid in these patients when first line treatments are intolerable, ineffective, or no longer effective due to dose-limiting side effects (8).

Defined as pain lasting longer than three months, chronic pain may affect one out of three patients at some point during their life. Medications used to treat chronic pain include non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen, antidepressants, benzodiazepines, anticonvulsants, clonidine, corticosteroids, muscle relaxants, and narcotic analgesics. Relaxation of prescribing guidelines, long half-life, and low price have made methadone an attractive alternative to higher priced sustained-release opioid preparations for treating chronic pain (9).

### Toxicity

*Side effects:* Methadone exhibits a side effect profile typical of most narcotic analgesics. Effects include dizziness, respiratory depression, clouded thinking, nausea and vomiting, pruritus, and constipation. Depression of respiratory control remains the side effect with the highest potential for adverse events. This is particularly true for the naive or casual user who lacks the protective effects of nociceptive input from pain pathways or tolerance (9).

*Driving:* Stout and Farrell recently published a review of the effects of opioids on human performance and behavior that included 21 clinical and 6 epidemiological studies of methadone (2). In the clinical studies, methadone adversely affected the ability of opiate-naive subjects to perform a variety of tests used to measure fitness for driving. As expected, these subjects experienced loss of visual acuity, slowing of simple and complex reaction times, and poor coordination.

More interesting results were obtained from studies of MMT patients and opioid-abstinent controls who were former heroin users. In most of these studies, the MMT patients performed as well as controls on a variety of tests. In studies that found significant variance between groups, the differences were determined to be psychological and socio-demographic rather than methadone-related.

In the epidemiological studies, two research groups found no difference in traffic violations between MMT patients and controls while a third study showed an increase in speeding violations but no difference in the crash rate. Studies of toxicological analyses from 1882 driver fatalities and 1194 drug-influenced drivers showed a methadone prevalence rate of 0.1% and 1.1%, respectively. A third study of 462 traffic-related cases revealed that methadone was the third most frequent finding in drug-influenced drivers and the fifth most frequently detected drug in traffic crashes.

**Overdose and death:** An increase in deaths attributed to methadone has been reported recently in the United States. A number of states, including North Carolina, Florida, Maryland, and Maine, have reported epidemic increases in methadone-related deaths since 1997. Table 3 details these increases (6, 10, 11). Similarly, DAWN data reports a 230% increase in mentions of methadone from emergency departments since 1994, with a 37% increase occurring between 2000 and 2001 (12). According to the Drug Enforcement Administration, drug diversion units at the state and federal level are also reporting unprecedented seizures of illegal methadone (13).

The major reasons for these overdoses are the overuse of methadone prescribed for the relief of chronic pain and diversion by the casual drug user. In Maine, the methadone in the drug deaths from 2002 was mostly in the liquid form, which indicates that the methadone was from MMT programs (6). Deaths associated with casual or intermittent abuse of methadone are not surprising, as deaths have been reported after ingestion of 50 mg or less of methadone in non-tolerant individuals (3).

Also not surprising is an increase in deaths after relaxation of rules regarding "take home" methadone from MMT programs. Similar increases in methadone deaths have occurred in Australia, Scotland, and England after public health changes that increased prescribing of methadone or allowed MMT patients to take home greater amounts (1).

**Interpretation of drug levels:** A number of studies have concluded that there is no significant differ-

ence between methadone blood levels in decedents whose death was attributed to methadone toxicity and those in which methadone was an incidental finding. One such study compared methadone concentrations between decedents whose death was due solely to methadone poisoning and a group of controls composed of living MMT patients. The blood methadone concentrations of the two groups were not statistically different, with the average of the decedents and MMT patients being 0.28 mg/L (range, 0.06–3.1 mg/L) and 0.11 mg/L (range, 0.03–0.56 mg/L), respectively (2).

To determine whether EDDP concentrations or EDDP/methadone ratios could distinguish between acute and chronic use of methadone, Karch and Stephens conducted a retrospective review of 3317 medical examiner cases, of which 38 were methadone positive. Seventeen of these decedents had methadone-poisoning deaths and in the remainder methadone was an incidental finding. Blood and urine concentrations of methadone and EDDP and methadone/EDDP ratios were not statistically distinguishable between groups (7). Interpretation of blood concentrations may also be complicated by post-mortem redistribution. In a study involving 111 methadone-positive decedents, multiple site sampling in 26 cases revealed that methadone concentrations from different sites could vary by a factor of two (2).

### Case studies

**Acute methadone toxicity:** A 17-year-old white female was found dead by her boyfriend two hours after she was observed to be drooling and wheezing. The history given to investigators by the boyfriend was notable for the ingestion of six Valium on the evening prior to her demise. Due to the unexpected nature of the death and circumstances indicating drug overdose, an autopsy was ordered.

Analysis of heart blood revealed the presence of diazepam (0.90 mg/L), nordiazepam (0.70 mg/L), methadone (0.61 mg/L), and butalbital (trace). Autopsy findings were consistent with drug overdose, with pulmonary edema the most significant finding.

Further investigation disclosed that the decedent's boyfriend and his stepmother were both treated for methadone toxicity at the local emergency department (ED). A reconstructed timeline of events revealed an illegal purchase of methadone by the boyfriend's stepmother in the parking lot of a local MMT center the evening prior to the decedent's demise. Several hours later the stepmother was taken to the ED where she was treated for methadone intoxication. In her absence, the stash of methadone was discovered and consumed by the decedent and

**Table 3. Increases in methadone-related deaths by state**

State	Study years	% Increase	Reference
Florida	1998–2001	1850	11
Maine	1997–2002*	450	6
Maryland	1997–2001	1000	10
N. Carolina	1997–2001	800	6

\* Includes only first half of 2002.

her boyfriend. After reporting her death, the boyfriend, who was observed to be ill during the interview, was admitted to the local ED and treated for methadone overdose.

*Death during initiation of methadone therapy for chronic pain:* A 38-year-old man with a history of chronic back pain and morbid obesity was prescribed methadone five days prior to death. Previous treatment for back pain included nonsteroidal anti-inflammatory drugs and physical therapy. His pain was not adequately controlled with this regimen, so a methadone prescription was written for 30 mg per day, the large amount due to his size.

Two days after beginning methadone therapy the decedent was found slumped in his bedroom doorway with shallow respiration and decreased consciousness. He was transported to the local ED where he was diagnosed with acute methadone intoxication and treated with naloxone. After several hours of observation and marked improvement in respiration, consciousness, and orientation, he was released with a suggestion to call his physician concerning his methadone prescription. His physician, via telephone, advised him to reduce his methadone intake to 15 mg per day. On day three and four he continued to experience marked drowsiness despite the change in dose. He was found dead in bed the morning of day five.

Autopsy findings were significant for morbid obesity and pulmonary edema. Microscopic analysis of sections from the lung revealed the presence of diffuse pneumonia. Specimens submitted for toxicological analysis included blood from the aorta and femoral vein and 50 grams of liver. The laboratory conducted routine analyses for drugs of abuse and organic acids, bases, and neutrals. Methadone and a trace amount of diphenhydramine were the only drugs detected. Quantification of methadone in the specimens submitted to toxicology resulted in the following: aorta blood, 0.11 mg/L; femoral blood, 0.06 mg/L; and liver, 2.1 mg/Kg. A pill count of his methadone prescription was consistent with the detailed history provided by the family.

### Case discussion

Both cases illustrate the importance of a complete and thorough medical examiner investigation. Wheezing, drooling, drowsiness, and snoring are symptoms of respiratory depression that may be noticed by family members or other witnesses prior to death. Autopsy findings will usually be non-specific with pulmonary edema as the most consistent finding. A diffuse or micro-pneumonia may also be detected and is indicative of severe respiratory depression. While not observable in Case 1 due to a lack of

specimens, Case 2 illustrates the post-mortem redistribution of methadone.

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## Assay Performance Must Be Defined & Verified Regularly

By David A. Armbruster

Good laboratory practice dictates that the key performance characteristics of assays be defined and verified periodically. This process has become a formal requirement under the federal government's Clinical Laboratory Improvement Amendments (CLIA) and the College of American Pathologists (CAP) Laboratory Accreditation Program (LAP).

CLIA sets specific requirements for the periodic calibration or calibration verification of assays, and the CAP has introduced a requirement for laboratories to define the analytical measurement range (AMR) and the clinical reportable range (CRR) for assays. There has been some confusion about the concepts of AMR and CRR, and how they relate to calibration and calibration verification. This article attempts to explain the CAP's requirement for AMR and CRR and to clarify its relationship to calibration and calibration verification. More detailed information is available on the CAP website (1, 2, 3).

### Analytical measurement range

The AMR depends on the number of calibrators used in an assay's calibration curve, but it is not always strictly defined by the calibrators. Immunoassays typically have nonlinear calibration curves. Immunoassays include a low-concentration calibrator (usually zero), a high-concentration calibrator, and as many intermediate calibrators as necessary to properly define the calibration curve. A curve-fitting program is used to define the analytical response from calibrator to calibrator, with some portions of the curve being relatively "steep" and others being relatively "flat."

For immunoassays, the AMR is the concentration range from the lowest calibrator to the highest calibrator. The relationship between the analyte concentration and the analytical signal at concentrations above the high calibrator is not defined. Specimens with higher concentrations cannot be measured unless they are diluted and brought within the AMR.

Tests other than immunoassays usually have linear calibration curves. A low calibrator (usually zero) and a high calibrator are used, but intermediate calibrators may not be used because only two points are required to define a straight line. The assumption is that the analytical response is linear between the low and high calibrator and that the linear relationship extends beyond the high calibrator. Of course, linearity will fail to hold beginning at some high

analyte concentration. The AMR may encompass analyte concentrations well above the high calibrator, but samples must be tested at higher concentrations to clearly define the AMR.

Determination of the AMR is analogous to the traditional linearity study. The purpose of the experiment is to measure the "analytical sensitivity" of the assay (which is the change in analyte concentration per change in analytical signal or the slope of the calibration curve) and determine the concentration range over which the analytical sensitivity remains constant (that is, the linear portion of the reaction curve). In the case of an immunoassay or any non-immunoassay for which the calibration curve is nonlinear, the analytical sensitivity isn't constant over the entire calibration curve. But, for any given segment of the calibration curve, the analytical response and the change in analyte concentration should be proportional. The AMR is determined or verified when the proportionality of changes in analyte and signal is confirmed.

The AMR study must test samples that span the anticipated or stated AMR. Ideally, the samples should be prepared in the same matrix (for example, serum, plasma, or urine) as patient specimens. A traditional approach is to mix differing proportions of high and low samples to produce a series of samples covering the AMR. If sufficient volumes of a zero-concentration sample and of a suitably high-concentration sample are available, they can be used to prepare intermediate concentration samples. Linearity samples, such as those available from the CAP or other suppliers, are another source of AMR samples (assuming that the samples respond appropriately, mimicking patient specimens, with the given methodology).

Standard EP6 (Linearity of Quantitative Analytical Methods) from the National Committee for Clinical Laboratory Standards recommends using five samples of different concentrations (4), but the CAP accepts a minimum of three concentrations. CAP recommends that the low sample be within 50% of the lower limit of the AMR and the high sample be within 10% of the upper limit of the AMR (obviously, the closer to the actual limits, the better). As with a traditional linearity study, the target concentrations of the samples are plotted on the X-axis and the observed concentrations are plotted on the Y-axis. A straight line relationship for the data points indicates a proportional analytical response for the different concentrations of analyte. The AMR is defined by the linear portion of this graph. Exactly *how* the linear relationship is judged (such as visually or by some mathematical calculation) is left to the discretion of the laboratory.

The CAP and CLIA require the AMR to be validated every six months. However, AMR validation is not required if the assay is recalibrated at least every six months using appropriate calibrators to represent the low, mid, and high ranges.

### Clinical reporting range

The CRR differs from the AMR in that it represents an assay's reportable range for a diluted or concentrated specimen; that is, it represents an expanded AMR. For example, if the AMR for an assay is 0–100 units, and if it has been demonstrated that an appropriate dilution, for example, 1:10, of a high-concentration specimen will bring it into the AMR of 0–100 units, then the CRR is 0–1,000 units. Laboratories are expected to define the standard dilutions to be used for assays and to validate that those dilutions produce results falling within the previously defined AMR for specimens that have not undergone any manipulation.

The CAP allows an exception for the CRR if a laboratory's policy is to dilute concentrated specimens until they are within the AMR. To illustrate with a toxicology example, a valproic acid immunoassay may have an AMR of 0–150 µg/mL (based on the low and high calibrators). If a laboratory requires a 1:2 dilution of any specimen greater than 150 µg/mL, the CRR is 300 µg/mL. Any result greater than 300 µg/mL is reported as such.

The new revision of LAP inspection checklists replaces linearity studies with AMR validation. As previously required by CLIA and the CAP, a calibration or calibration verification must be performed every six months (calibration every six months or more frequently obviates the need for calibration verification as long as three or more calibrators that span the AMR of the test are used). AMR verification is also required every six months, using a minimum of three levels (low, mid, and high). As noted previously, if the number and concentration of calibrators is appropriate, and if calibration is performed at least every six months, AMR is not required because the calibration serves the same purpose.

The revised automated general chemistry inspection checklist, AGC.23120, requires the AMR to be defined for all analytes so that results exceeding the limits are reviewed, and specimens reassayed if necessary, before reporting (3). The note accompanying the checklist question defines AMR as “the range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment not part of the usual assay process.” Values above or below the AMR may be reported without retesting if the values are reported as less than the lower limit or greater than the upper

limit of the AMR. The CRR is defined as the “range of analyte values that a method can report as a quantitative result, allowing for specimen dilution, concentration, or other pretreatment used to extend the direct AMR.” Helpful examples using the AMR and CRR for human chorionic gonadotropin and aspartate transaminase assays are included in the checklist.

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**Q** In laboratories that are not subject to mandatory immunoassay cutoff concentrations, how should appropriate cutoff concentrations for drugs of abuse assays be determined?

*Answered by Sarah Kerrigan*

**A** The cutoff concentration should be well above the limit of detection of the assay. Ideally, the cutoff should be in a region of the dose-response curve where there is a large change in signal per unit of concentration (that is, where the analytical sensitivity is at a maximum). Choosing a point at the steepest part of the curve ensures that there is good separation between a positive and a negative sample at the cutoff concentration. Cutoffs should not be selected at concentrations that coincide with a plateau in the dose-response curve.

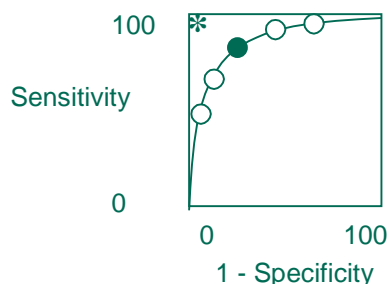
The most appropriate cutoff concentrations are determined by using case samples that have been analyzed using a confirmatory technique such as gas chromatography/mass spectrometry so they are known to be either true-positive or true-negative results. These samples can then be tested using the drugs-of-abuse immunoassays over a range of potential cutoff concentrations.

The total number of false-positive (FP), false-negative (FN), true-positive (TP) and true-negative (TN) results can be determined at each proposed cutoff value. This allows sensitivity (probability of correctly identifying a positive sample) and specificity (probability of correctly identifying a negative sample) to be determined as follows:

$$\text{Sensitivity} = (\text{TP} \times 100) / (\text{TP} + \text{FN})$$

$$\text{Specificity} = (\text{TN} \times 100) / (\text{TN} + \text{FP})$$

The cutoff concentration should be selected in accordance with acceptable thresholds for sensitivity and specificity in the context of institutional or regulatory requirements and the type of casework performed. Alternatively, cutoff concentrations can be selected through the use of receiver operating characteristic (ROC) plots, which are formed by plotting sensitivity vs. (1 - specificity). Each cutoff concentration tested is represented by one data point on the ROC curve. The cutoff concentration that provides optimum sensitivity and specificity is represented by the data point closest to the asterisk on the ROC plot below (darkened circle):



Selection of the immunoassay cutoff concentration at this value should result in the minimum number of false-positive and false-negative results. If the positive predictive value and negative predictive value are needed, they can be calculated as follows (where "prevalence" is the percentage of true-positive samples in an examined population).

Positive predictive value is:

$$\frac{\text{Sensitivity} \times \text{Prevalence}}{(100 - \text{Specificity}) \times (100 - \text{Prevalence}) + (\text{Sensitivity} \times \text{Prevalence})}$$

Negative predictive value is:

$$\frac{\text{Specificity} \times (100 - \text{Prevalence})}{(100 - \text{Sensitivity}) \times \text{Prevalence} + [\text{Specificity} \times (100 - \text{Prevalence})]}$$

### Suggested Reading

1. Ferrara et al. Drugs of abuse testing in urine: statistical approach and experimental comparison of immunochemical and chromatographic techniques. *J Anal Toxicol* 1994;18(5):278-91.
2. Zweig et al. Receiver operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;39:561.

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*Clinical & Forensic Toxicology News* is an educational service of the forensic urine drug testing (FUDT) program. The FUDT program, cosponsored by the American Association for Clinical Chemistry and the College of American Pathologists, includes three components: FUDT accreditation, the FUDT proficiency testing survey, and this newsletter. The accreditation program is the responsibility of the CAP. The surveys are sponsored jointly by AACC and CAP. The newsletter is published quarterly by the American Association for Clinical Chemistry, Inc., 2101 L St., N.W., Suite 202, Washington, DC 20037, (800) 892-1400 or (202) 857-0717, [cftnews@aacc.org](mailto:cftnews@aacc.org).

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