Determination of monoethylglycinexylidide by fluorescence polarization immunoassay in highly icteric serum samples: modified precipitation procedure and HPLC compared

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Hyperbilirubinemia, which frequently occurs in severe liver disease, interferes with the fluorescence polarization immunoassay (FPIA) monoethylglycinexylidide (MEGX) assay manufactured by Abbott Diagnostics. Because the MEGX test is particularly helpful in this clinical situation, strategies have been developed to overcome this problem. Precipitation of serum with the Abbott Digoxin II precipitation reagent eliminates bilirubin. Therefore, we compared FPIA results after precipitation of 81 icteric samples from 27 MEGX tests to results obtained using a validated HPLC method. The precipitation did not substantially alter the performance characteristics of FPIA: detection limit, 8 µg/L; between-days imprecision, 5.3–6.2%; recovery, 102–104% (50–200 µg/L). This pretreatment of serum did not eliminate all interference, and only a poor correlation was observed between serum MEGX concentrations measured with HPLC or modified FPIA ($r^2 = 0.46; S_{y|x} = 20.0 \mu g/L$). In contrast, MEGX formation values calculated by subtraction of the predilucaine MEGX concentration were in close agreement ($r^2 = 0.98; S_{y|x} = 2.3 \mu g/L$). Because only MEGX formation is clinically relevant, this modified FPIA procedure offers a simple and rapid alternative to HPLC.

The hepatic conversion of lidocaine to the metabolite monoethylglycinexylidide (MEGX) is primarily catalyzed by the cytochrome P450 enzyme 3A4 in humans (1). This metabolic capacity is the basis of the MEGX test, a blood flow-dependent, real-time liver function test in which serum MEGX concentrations are determined 15–30 min after an intravenous bolus of lidocaine (1 mg/kg body weight). The test is easy to perform, and no severe adverse side effects have been reported since the introduction of the test (2–4). Presently, there is a large and convincing body of evidence documenting the usefulness of the test in a wide variety of clinical situations with impaired liver function. MEGX formation 15 or 30 min after an injection of lidocaine is a sensitive indicator of the severity of end stage liver disease (ESLD) (5, 6). Prospective studies have revealed that MEGX test results are of prognostic value for assessing and monitoring adult as well as pediatric liver transplant candidates (7, 8). In the early postoperative period after orthotopic liver transplantation (OLT), serial monitoring of liver function with the MEGX test is predictive of the short-term outcome of liver graft recipients (7–10). A recent review of several studies in graft recipients and ESLD patients (11) concluded that a MEGX concentration <15–25 µg/L is associated with severe impairment of liver function. The MEGX test has also proven useful in other clinical situations. In critically ill patients at high risk for development of multiple organ failure, low MEGX formation values are an early predictor of a fatal outcome (12, 13).

An automated fluorescence polarization immunoassay (FPIA; Abbot Diagnostics Division, Chicago, IL) is widely available for MEGX determination in serum. The assay has a detection limit $<3 \mu g/L$, is easy to perform, and allows MEGX determination within ~20 min, which is important for evaluating liver grafts. Many of the clinical settings mentioned above, particularly in the early post-
transplant period, are associated with a pronounced hyperbilirubinemia that is known to interfere with analytical measurement by FPIA, causing a substantial increase in background fluorescence (instrument gives an error reading of “MX BK”). To overcome this limitation, serial dilution with system buffer has been proposed (6). An alternative procedure uses the pretreatment of serum with the precipitation reagent from the Abbott Digoxin II assay (14), which eliminates protein as well as visible bilirubin from the specimen. These modified procedures have not, however, been validated by comparison with an established HPLC method. We have now compared the MEGX test results obtained after the precipitation of hyperbilirubinemic serum samples with the corresponding results obtained with an HPLC procedure (15). The original precipitation procedure (14) was hampered by the need for two separate calibration curves, because the sample volume is modified by the reprogramming of the TDx (Abbott Diagnostics) assay. This approach increases the costs because a second calibration curve must be run, and quality assurance is necessary for two (modified and unmodified) MEGX assays. In addition, measuring both untreated and treated serum samples in the same run, is not possible. This pretreatment procedure for the TDx was, therefore, simplified to allow measurement with the same calibration curve used for untreated samples.

Patients, Materials, and Methods

Patients

Eighty-one serum specimens from 27 MEGX tests, performed on 10 patients after OLT and 4 patients with ESLD, were used for the comparison. All samples produced an error reading (“MX BK”) when the MEGX concentration was measured with the FPIA without prior precipitation. The allowed maximum blank fluorescent polarization rate was set to 3500 (arbitrary units), as proposed in the manufacturer’s manual. Serum bilirubin concentrations in the samples ranged from 140 to 816 μmol/L.

Pretreatment for FPIA

Precipitation. Two hundred microliters of serum and 200 μL of Digoxin II precipitation reagent were manually pipetted into a 1.5-mL centrifugation cup, thoroughly mixed for 30 s, and allowed to stand for 10 min. After centrifugation (5 min, 10 000g), the supernatant was used directly for MEGX determination with a TDx system. The measured values were multiplied by two for calculation of the final result.

Dilution. Specimens were diluted with TDx system buffer until the blank was below the limit mentioned above. Results were obtained by correcting the system readings by the necessary dilution factor.

Calibration and control materials were purchased from Abbott Diagnostics, the manufacturer of the FPIA.

HPLC

HPLC measurements were performed as described elsewhere (15). Briefly, 500 μL of serum were mixed with an internal standard and used for extraction of MEGX. Before injection into the HPLC column, both the internal standard and MEGX were derivatized with a fluorophore. Reversed-phase HPLC was carried out with a Shimadzu LC-10A (Shimadzu), and fluorescence peak areas were used for result calculation. The between-days imprecision of this assay ranged from 3.8% to 12.7% (2.5–125 μg/L), with a dynamic quantification range from 2.3 to 250 μg/L. Bilirubin does not interfere with this HPLC procedure because it is extracted to <5%, and furthermore, does not react with the derivatization reagent.

MEGX Test

The test was performed as proposed originally (2). Briefly, 1 mg of lidocaine per kg of body weight was injected intravenously over 2 min. Blood samples (5 mL) were withdrawn from the contralateral cubital vein or from a catheter before the injection, as well as 15 and 30 min after the end of injection. MEGX formation (15 and 30 min) was calculated by the subtraction of the prediloxane MEGX concentration in serum from the values obtained after 15 and 30 min.

When the MEGX concentration in prelidocaine samples was below the detection limit of either method, no subtraction was made.

Interference Studies

To gain an insight into the possible nature of the interferences, drugs that were administered in those patients with samples showing high interferences and some drugs that are often administered in patients after OLT and with ESLD were tested in vitro. The drugs were mixed with drug-free sera, as well as with MEGX control sera, at the concentrations indicated in the Results section.

Statistics

For method comparisons, the nonparametric regression method described by Passing and Bablok (16) was used. The estimates for slope and intercept are given, with the 95% confidence intervals given as a measure of agreement in parentheses. Pearson’s r and Kendall’s τ are also included. The dispersion of the residuals was calculated as the standard deviation of the residuals (Syx) from the standard principle component method. Calculations were carried out with dedicated software (EVAPAK, Ver. 2.08; Boehringer Mannheim).

Results

The performance characteristics of the precipitation method are given in Table 1. Precipitation pretreatment did not negatively influence the performance of the FPIA procedure. The detection limit determined according to Kaiser (17) was 8 μg/L, compared with 3 μg/L without pretreatment. The modification of the assay led to a
slightly increased day-to-day imprecision compared with the use of control samples without precipitation (Table 1). However, because there was no deviation from the target values of the control material after precipitation, multiplication by 2 yielded the correct results after pretreatment.

To evaluate the precision with patients samples, 21 serum specimens (mean MEGX concentration, 33.1 μg/L) were retested. The average deviation from the means of the duplicates was 2.2% (range, -18% to 11%; SD, 9.5%).

In a subset of samples, serial dilution with the TDx system buffer was investigated. This approach yielded unacceptable results for MEGX concentrations, as shown by the poor correlation with HPLC (x-values; with prelidocaine MEGX concentrations included (95% CI in parentheses), n = 21, r = 0.51, τ = 0.41, y = -1.37(-36.0 -19.0) + 1.49(0.68 -3.02)x, $S_{yx} = 24.4$ μg/L; without prelidocaine values, n = 14, r = 0.54, τ = 0.57, y = -9.25(-44.8 -19.1) + 1.94(0.73 -3.33)x, $S_{yx} = 25.8$ μg/L). Subtraction of basal values to calculate MEGX formation did not improve the comparability of FPIA- and HPLC-based measurements (Fig. 1). We therefore decided not to pursue this strategy further.

The MEGX concentrations measured in the study group 15 and 30 min after the lidocaine bolus, using the modified precipitation/FPIA procedure, also displayed a wide scatter compared with HPLC. The correlation was poor, and both over- and underestimation were observed (Fig. 2). This also held true if prelidocaine values (0 min) were included in the calculation (n = 67, r = 0.57, τ = 0.45; y = 1.55(-3.92 -5.79) + 1.49(1.11 -1.87)x, $S_{yx} = 19.7$ μg/L). The median difference in MEGX concentrations between the FPIA and HPLC methods was 7.0 μg/L, ranging from -38.5 to 111 μg/L. There was no apparent

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### Table 1. Day-to-day imprecision of untreated and precipitated manufacturer MEGX controls.

<table>
<thead>
<tr>
<th>Target value</th>
<th>10&lt;sup&gt;a&lt;/sup&gt;</th>
<th>25&lt;sup&gt;a&lt;/sup&gt;</th>
<th>50</th>
<th>100</th>
<th>200</th>
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<td>99.3</td>
<td>197.5</td>
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<td>3.4</td>
<td>7.0</td>
<td>1.3</td>
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<td>3.5</td>
<td>3.5</td>
<td>11.5</td>
<td>6.7</td>
<td>5.4</td>
<td>5.3</td>
<td>6.2</td>
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<sup>a</sup> The control material with a target of 50 μg/L was diluted with TDx System buffer (1 mL of control material + 1 mL of buffer or 1 mL of control material + 4 mL of buffer) to achieve these target values.
correlation between this difference and the underlying bilirubin concentration in the samples ($r^2 = 0.096$). Prolidocaine samples showed an apparent MEGX concentration in 23 of 27 samples when measured with FPIA and in 13 of 27 samples when measured with HPLC. Because the HPLC procedure has no known interference, the MEGX in pretest serum samples is probably because of previous lipocaine administration to those patients who showed measurable MEGX serum concentrations with HPLC. The increased apparent MEGX serum concentrations measured with FPIA, both before and after lipocaine injection, were not the results of high lipocaine concentrations in the samples, because the lipocaine concentration did not exceed 4.5 mg/L in any of the 81 samples included in this study. Significant cross-reactivity with the MEGX FPIA was observed only at lipocaine concentrations $>10$ mg/L.

In contrast to the lack of comparability of MEGX serum concentrations, MEGX formation values, which were obtained by subtracting the prelipocaine MEGX value from the 15- and 30-min MEGX test results, revealed almost identical results with both test systems (Fig. 3). When stratified according to 15- or 30-min formation values, regression analyses showed that the FPIA and HPLC values of 30-min MEGX formation are in somewhat closer agreement compared with the 15-min values ($r = 0.992$, $\tau = 0.87$, $S_{y|x} = 1.95 \mu g/L$ vs $r = 0.980$, $\tau = 0.65$, $S_{y|x} = 2.6 \mu g/L$).

Several drugs that are administered to those patients who showed a high bias between MEGX concentrations measured with HPLC and FPIA were investigated as possible causes of interference. The list of drugs tested and the maximum concentrations used were as follows: mycophenolic acid (50 mg/L), fomotidine (200 mg/L), trimetoprim/sulfamethoxazole (800 mg/L trimetoprim and 4 g/L sulfamethoxazole), antithrombin III (2500 IU/L), aciclovir (250 mg/L), cyclosporine A (50 mg/L) N-acetylcysteine (500 mg/L), and tacrolimus (140 µg/L). In addition to those drugs, human albumin (9.5 g/L) and bile acids (2.4 mmol/L) were also investigated.

None of the tested substances revealed a positive or negative deviation $>5\%$ from the target value when added to the MEGX control serum (100 µg/L), nor did they show a MEGX concentration above the detection limit when tested in drug-free serum up to the concentration indicated in parentheses.

**Discussion**

Interference from bilirubin is a shortcoming of the FPIA MEGX assay performed on the Abbott TDx system for monitoring liver function in ESLD. This is especially true during the early postoperative period after OLT, because $>30\%$ of liver recipients may suffer from icteric cholestasis during the first weeks after OLT (9). Icteric cholestasis after OLT, however, is transient in most cases and does not influence the outcome of transplantation. Nevertheless, the MEGX test has proven to be very helpful in these clinical situations for the management of disease (9, 11), because after OLT as well as in ESLD, the MEGX test result is of particular prognostic value and is superior to static liver function tests (7, 9–11, 18). In addition, the MEGX test has gained increasing importance in the management of severely injured patients, to assess the risk of multiple organ failure with fatal outcome (12, 13). Strategies have been described to overcome the problem of bilirubin interference in the FPIA assay (6, 14), but none have been validated against an interference-free method, such as HPLC. We have now compared a pretreatment procedure that uses the Digoxin II precipitation reagent and consecutive FPIA measurement with a recently developed highly specific, sensitive HPLC-fluorescence assay (15). The results show that the adapted precipitation method is capable of accurately quantifying MEGX formation in humans, using the FPIA procedure even in the presence of high bilirubin concentrations. The pretreatment of serum samples with the Abbott Digoxin II precipitation reagent revealed reproducible results with an acceptable precision and recovery. Because we do not increase the sample volume of the MEGX TDx assay (14), it is possible to perform both the original and the modified tests on one system with one calibration curve, which has the advantage of reducing costs compared with other approaches. However, care should be taken in interpreting MEGX concentrations determined with this method because not all interfering substances are

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![Figure 3](https://example.com/figure3.png)

*Fig. 3. Comparison of MEGX formation values determined 15 and 30 min after lipocaine injection [with basal value (0 min) subtraction], using HPLC or using FPIA after precipitation (n = 54, $r = 0.99$, $\tau = 0.77$, $y = 0.74(0.14 - 1.30) + 1.00(0.92 - 1.08)x$, $S_{y|x} = 2.3 \mu g/L$). The solid line represents the estimated regression; the dashed line represents the line of identity.*
eliminated by this procedure. This phenomenon was illustrated by the wide deviation between the serum MEGX concentrations determined with either HPLC or FPIA, with both over- and underestimation by FPIA. Only when MEGX concentrations of the samples withdrawn before lidocaine injection were subtracted from the 15- and 30-min values to calculate MEGX formation rate was a favorable agreement found. Obviously, the cause of interference in the FPIA could not be totally eliminated in all cases, but in the patients tested, it remained constant during the 30 min of the MEGX test, yielding correctly calculated MEGX formation values. These interferences were apparently not the results of several drugs that are given concomitantly, because they did not produce a deviation from the target value when mixed with control sera. Proteins can be excluded as sources of interference, because they are almost completely eliminated by the precipitation procedure. Several substances, such as fluorescein and mercuric chrome, are known to cause high fluorescence signals and have been shown to interfere with TDx assays. However, such substances were not present in our samples and so could not explain the observed effects on the MEGX results. A large number of interfering substances are known for the digoxin TDx assay in patients with liver disease, and those can lead to a substantial overestimation of digoxin results. Endogenous substances that accumulate in these patients have been proposed as the cause of this interference. Because a large sample volume is used in the MEGX assay, which is, moreover, analytically highly sensitive, it can be speculated that small concentrations of endogenous interfering substances present in patients with liver dysfunction with hyperbilirubinemia may be sufficient to produce the observed bias from the HPLC procedure. However, the possibility that drug metabolites generated in vivo may be the causes of the interferences cannot be ruled out. Although in this investigation the cause of the interference appeared to be constant over the 30-min sampling period that was required for the MEGX test, changes within individual patients cannot be completely excluded.

However, because the MEGX formation values were not erroneous after precipitation in all the patients investigated, this procedure would appear to be generally acceptable in terms of clinical use. Only MEGX formation values, obtained after subtraction of the prelidocaine (0 min) values, are of interest for the assessment of liver function. All studies underlining the clinical usefulness of the MEGX test are based on formation rates, not on absolute concentration. In contrast, in a subset of samples, simple dilution with system buffer led to results on the TDx system that were not in as close agreement to HPLC as those seen with the precipitation pretreatment. This may also be caused by the high sample volume (10 μL) used by the TDx for the MEGX assay, with a higher risk of matrix effects compared with other TDx assays, which usually require sample volumes of only 2–3 μL.

In conclusion, the described precipitation method for the determination of MEGX formation with the FPIA is reliable for clinical use and easy to perform; it may, therefore, serve as an alternative to HPLC measurement in hyperbilirubinemic samples.

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