# TECHNICAL NOTE

*Ilkka Ojanperä,*[1] *M.Sc.; Erkki Vuori,*[1] *M.D.;*
*Raija Nieminen,*[1] *M.Sc.; and Antti Penttilä,*[1] *M.D.*

# Screening for Barbiturates in Vitreous Humor by the EMIT®-st™ Serum Enzyme Immunoassay

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**ABSTRACT:** In 16 medical examiner's cases, which were found to be barbiturate-positive by thin-layer chromatographic screening of the liver, blood barbiturate concentrations were determined by gas chromatography. The corresponding vitreous humor samples were screened by the enzyme multiplied immunoassay technique, the EMIT®-st™ serum barbiturate assay. By using the recommended dilution for detecting serum barbiturates, it was possible to detect barbiturates in vitreous humor at a toxic concentration. By using one fourth the amount of diluent, the barbiturates could be detected also at a therapeutic concentration. The EMIT-st assay proved to be useful in the screening for barbiturates in vitreous humor, a material that is readily available in forensic toxicology.

**KEYWORDS:** toxicology, barbiturates, vitreous humor, immunoassay

The qualitative EMIT®-st™ enzyme immunoassay (Syva Corp., California), which is a modification of the enzyme multiplied immunoassay technique, has proved to be useful in the screening for various drugs in clinical toxicology [*1*]. The main advantages of the EMIT-st assay are rapidity, small sample size, and the facility of handling the samples.

The EMIT-st system is designed for detecting drugs in serum, plasma, or urine. In forensic toxicology, however, serum cannot be obtained because of hemolysis. It has been shown that urine is available in only 35% of putrefied bodies, while vitreous humor is available in 70% of such cases [*2*]. The present study widens the scope of the EMIT-st system by demonstrating the method's usefulness in the screening for barbiturates in postmortem vitreous humor.

## Materials and Methods

Vitreous humor, liver, and femoral venous blood specimens were collected from medical examiner's cases at autopsy, and stored refrigerated. Blood samples from 16 cases, which

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[1] Assistant toxicologist, chief toxicologist, toxicologist, and associate professor and chief, respectively, Division of Forensic Chemistry, Department of Forensic Medicine, University of Helsinki, Finland.

were found barbiturate-positive by thin-layer chromatographic analysis of the liver, were analyzed quantitatively by gas chromatography. The corresponding vitreous humor samples were analyzed by the EMIT-st serum barbiturate assay.

For quantitative determination, the barbiturates were extracted from 5 to 10 mL of blood into dichloromethane, and the organic solvent was evaporated to dryness. The residue was dissolved in distilled water and extracted with dichloromethane. After separation and evaporating the organic solvent to dryness, the residue was reconstituted with ethanol and analyzed by gas chromatography using nitrogen selective detection. The column was a 1-m by 3-mm inside diameter (ID) Ni-column packed with 3% OV-17 on 100-120 mesh Gas-Chrom Q, and the column oven temperature was kept isothermally in the range of 150 to 210°C, depending on the compound analyzed.

The barbiturates were detected in vitreous humor by the EMIT-st serum barbiturate assay using the appropriate reagents and apparatus. The method has been described in detail elsewhere [*1*]. Three dilutions were made, if the specimen size was sufficient: 12 mL (Dilution A), 6 mL (Dilution B), and 3 mL (Dilution C) of distilled water were added to 200-μL portions of clear vitreous humor, and the mixtures were shaken vigorously for 30 s. Of the dilutions, 3050 μL were then transferred to the assay with a Finnpipette (Labsystems Corp., Finland).

## Results and Discussion

Table 1 shows the blood barbiturate concentrations and the EMIT-st results of the vitreous humor dilutions. The A dilution corresponds to the recommended EMIT-st serum barbiturate assay dilution, and the B and C dilutions are about two and four times more concentrated, respectively.

A logical connection between the EMIT results is evident. There is, however, one positive

TABLE 1—*The barbiturate blood concentrations compared with the vitreous humor EMIT-st results.*

| Case | Compound | | Blood, mg/L | Vitreous A | Vitreous B | Vitreous C |
|---|---|---|---|---|---|---|
| 1 | amobarbital | | 2 | − | − | . . . |
| 2 | pentobarbital | | <1 | − | − | − |
| 3 | pentobarbital | | 2 | − | + | + |
| 4 | pentobarbital | | 4 | + | + | + |
| 5 | pentobarbital | | 5 | + | + | + |
| 6 | pentobarbital | | 5 | + | . . . | . . . |
| 7 | pentobarbital | | 7 | + | + | . . . |
| 8 | phenobarbital | | <1 | − | − | − |
| 9 | phenobarbital | | 2 | − | − | − |
| 10 | phenobarbital | | 3 | − | − | − |
| 11 | phenobarbital | | 5 | − | +[b] | − |
| 12 | phenobarbital | | 6 | − | − | . . . |
| 13 | phenobarbital | | 9 | − | − | + |
| 14 | secobarbital and brallobarbital[a] | secob.<br>brallob. | 2<br>3 | − | + | + |
| 15 | secobarbital and brallobarbital[a] | secob.<br>brallob. | 14<br>14 | + | . . . | . . . |
| 16 | secobarbital and brallobarbital[a] | secob.<br>brallob. | 10<br>24 | + | . . . | . . . |

[a]Available in Finland as a combination preparate only.

[b]An anomalous result arising from an exceptionally low calibrator reading.

result (Case 11 B), which differs from expected. In this case the calibrator reading was exceptionally low (162) compared to that generally found (222-269).

It has been demonstrated that blood and vitreous humor barbiturate concentrations are approximately the same [3]. The present study is in good agreement with this, when the following EMIT-st sensitivity limits, given by the manufacturer, are taken into account: amobarbital $\leq 15$ mg/L, pentobarbital $\leq 6.0$ mg/L, phenobarbital 12 to 25 mg/L, and secobarbital 3.0 mg/L.

## Conclusions

The EMIT-st serum barbiturate assay was found to be useful in the screening for barbiturates in postmortem vitreous humor. By using Dilution C, 200 $\mu$L of vitreous humor + 3 mL of water, it was possible to detect many barbiturates not only at the toxic concentration, but also at the therapeutic concentration.

## References

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Address requests for reprints or additional information to
Ilkka Ojanperä
University of Helsinki
Department of Forensic Medicine
Kytösuontie 11
SF-00280 Helsinki, Finland