

Clinical Accuracy Study Methods and Test Data

This clinical study was conducted between March 27, 1995 and March 31, 1995. In the first part of this study, the Test-mate ChE Cholinesterase Test System was directly compared to the Boehringer Mannheim Cholinesterase Test System #450035 performed on their Hitachi model 704 Blood Analyzer (BM/H). The BM/H system was chosen as a "gold standard" because it is an FDA legally marketed product and specifically cited in the Federal Register. In the second part of this study, blood samples were dosed with paraoxon to produce cholinesterase inhibition caused by an organophosphorus compound. This two part analysis was designed to obtain the NCCLS (National Committee for Clinical Laboratory Standards) requisite samples in an ethical and practical fashion.

The study population was made up of normal blood donors from the Cincinnati, Ohio area, about one-half male and one-half female. Two-thirds of the samples were obtained at a surgical instrument company and the other one-third obtained from an apheresis center. This sample population of blood donors is representative of the blood samples that would be encountered when using the Test-mate ChE. In actual use, farm workers are tested to obtain a baseline cholinesterase value prior to being exposed to pesticides. The Cincinnati sample population is representative of this unexposed group. The farm workers are then periodically tested to monitor for a significant drop from their baseline value. Should the cholinesterase level of a worker drop substantially from their baseline, pesticide overexposure is indicated. The exposed group is modeled in this study by adding paraoxon to split samples of the population. This scientifically valid method was followed because the incidence of pesticide exposure among farmworkers is too low to collect enough data to be statistically significant, and because the normal ranges of plasma and erythrocyte cholinesterase are too narrow for definitive statistical comparisons.

Strict ethical informed consent procedures were followed throughout this study. The Deputy Director of Blood Services of the Hoxworth Blood Center, Daryl Everett, composed a description of this study which was read by each donor prior to their consent. Each donor was informed that two 10 μ L capillary tubes and one venipuncture tube of their blood would be taken for use in this research study along with their normal blood donation. After performing a copper sulfate hematocrit determination and checking the donor's blood pressure, the collection nurse drew the two capillary tubes of blood and immediately placed them into Test-mate ChE assay buffer tubes and shook them vigorously for the required 15 second period. Within 15 minutes these two assay tubes were analyzed by Dr. Magnotti of EQM Research for plasma cholinesterase, erythrocyte cholinesterase and hemoglobin using the Test-mate ChE. The room temperature varied between 17°C and 26°C when the samples were processed. Approximately 30 minutes after the capillary tubes were obtained, an EDTA (purple-top) collection tube of blood was taken along with the four unit blood donation. The purple top tube containing approximately 5mL of blood was split in half; one half was dosed with differing amounts of paraoxon (the active metabolite of the pesticide parathion) to produce inhibitions of plasma and erythrocyte cholinesterase ranging from

about 20% to 80% of normal activity. Paraoxon was chosen to dose samples, as it is a potent, stable, easily available, and well characterized inhibitor with fast inactivation yet slow reactivation kinetics, producing a well defined, stable level of inhibition comparable to that observed in real poisonings. From 45 donors, 90 total specimens (including those with added paraoxon) were thus obtained.

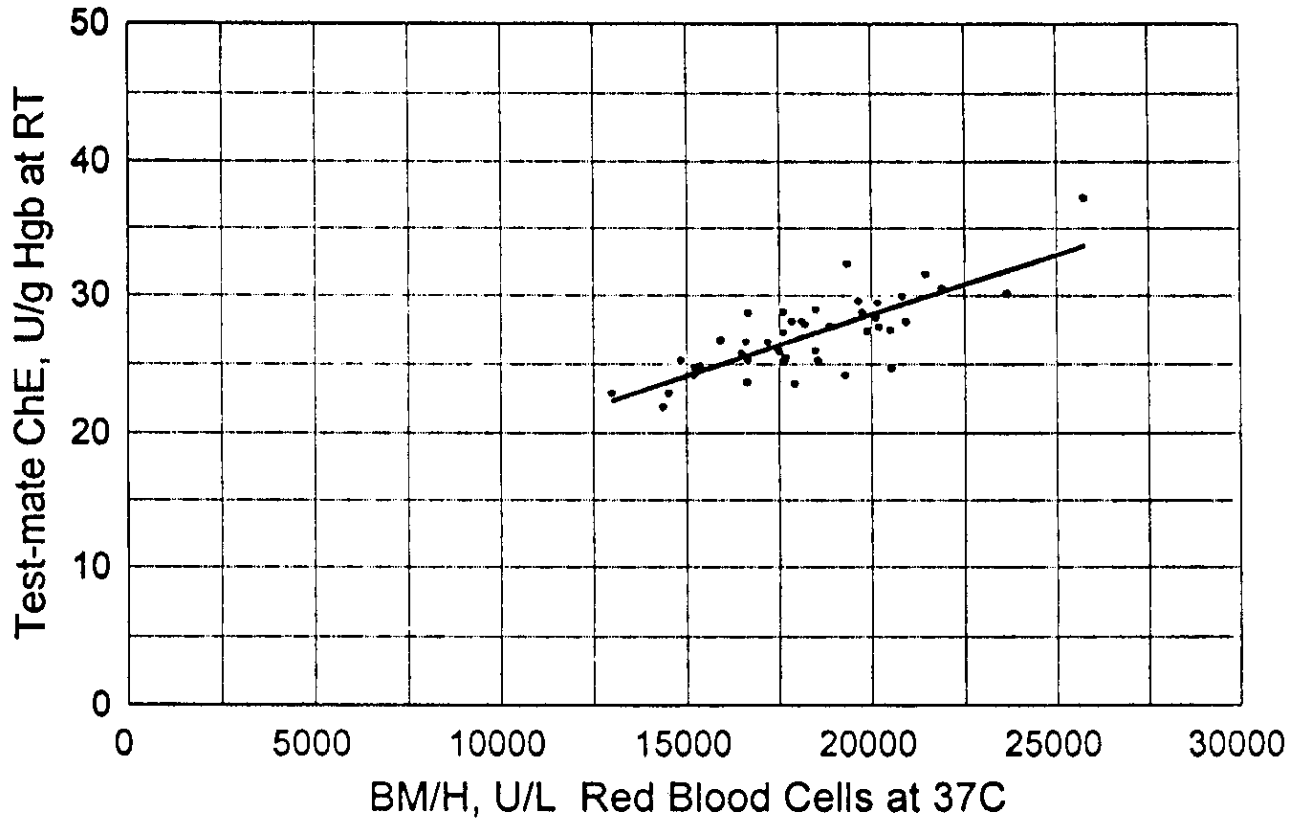
Samples of the 90 blood specimens were analyzed by the University of Cincinnati Hospital Toxicology Laboratory (certified by CLIA, CAP and JCAHO) to determine both erythrocyte and plasma cholinesterase levels using the BM/H system. Samples of the blood specimens were sent to the University of Cincinnati Hospital Hematology Laboratory for a complete blood count, including both hemoglobin and hematocrit, using a Coulter Blood Cell Counter. And finally, samples of the blood specimens were analyzed by Dr. Eberly of EQM Research for plasma cholinesterase, erythrocyte cholinesterase and hemoglobin using the Test-mate ChE system.

This was a double blind study. The University of Cincinnati did not release their results until after all analyses were completed. EQM Research was provided with the original printouts from the BM/H system and the Coulter Blood Cell Counter. Copies of the original data will be made available to the FDA upon request.

The test data from this study was analyzed using Quattro Pro 6.0 to produce plotted graphs and provide statistical parameters [see fig. 14-A, fig. 14-B, fig. 14-C, fig. 14-D and fig. 14-E].

AChE: Normal Population Analysis

Venipuncture vs. Fingerstick, respectively

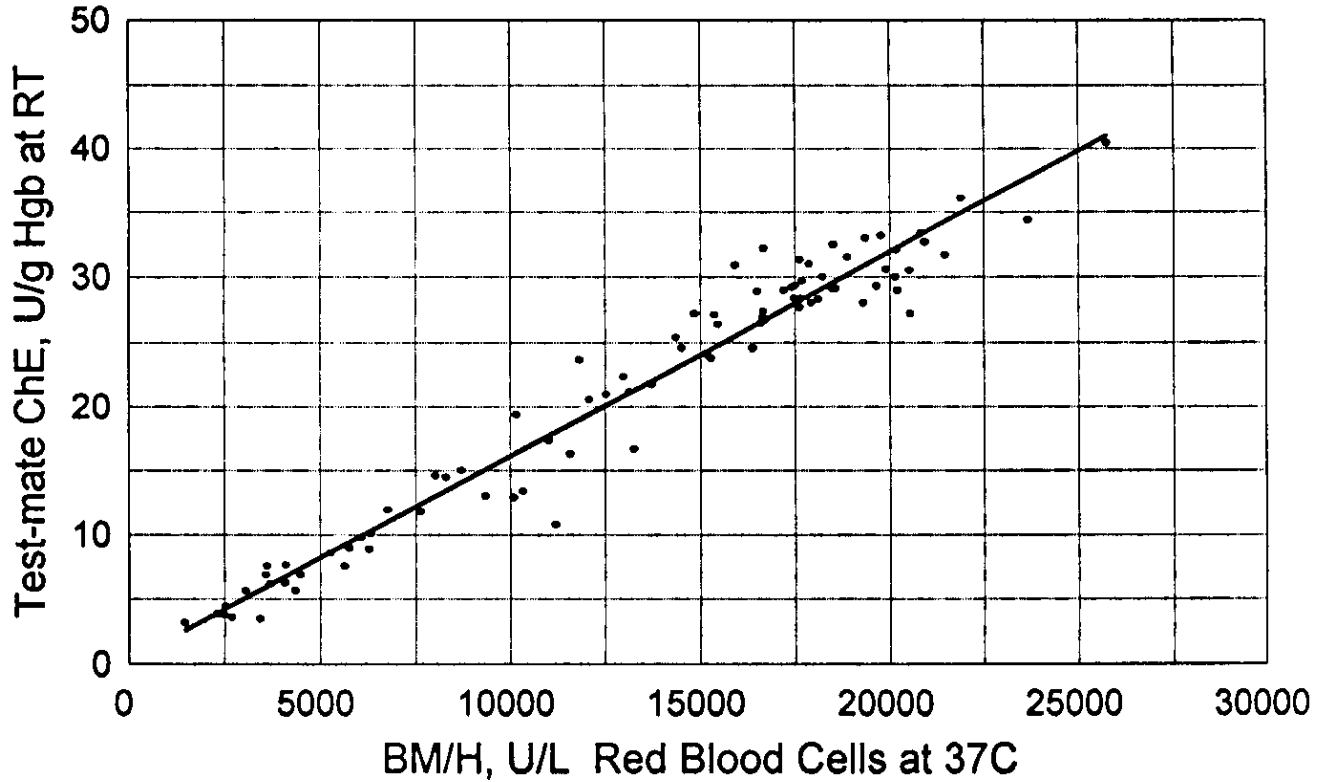


Regression Output:

Constant	10.75225
Std Err of Y Est	1.799962
R Squared	0.615397
No. of Observations	44
Degrees of Freedom	42
X Coefficient	0.000894
Std Err of Coef.	0.000109
Correlation Coef.	0.784472

Fig. 14-A

AChE: Same-Sample Analysis
Including Pesticide Spiked Samples



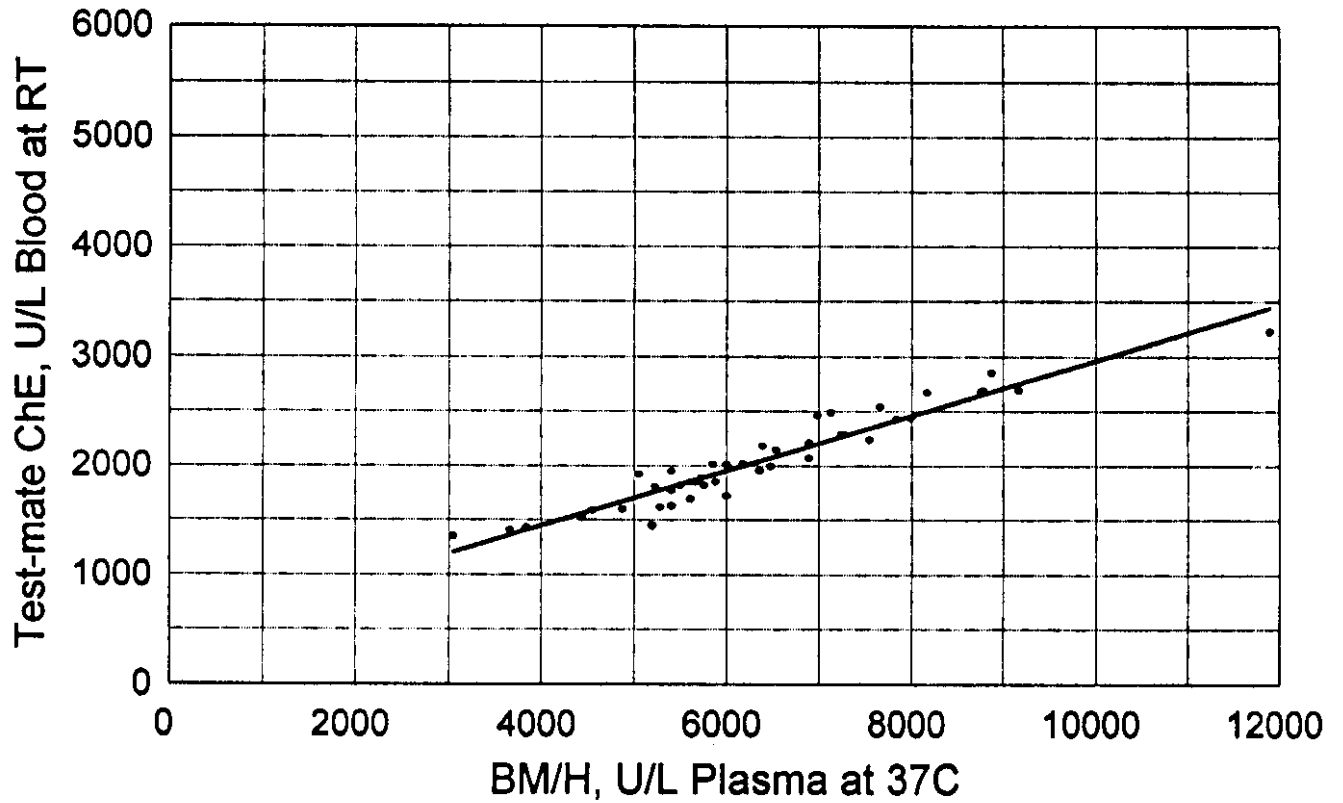
Regression Output:

Constant	0.3219
Std Err of Y Est	2.1595
R Squared	0.9556
No. of Observations	86
Degrees of Freedom	84
X Coefficient	0.001582
Std Err of Coef.	0.000037
Correlation Coef.	0.977548

Fig. 14-B

PChE: Normal Population Analysis

Venipuncture vs. Fingertick, respectively



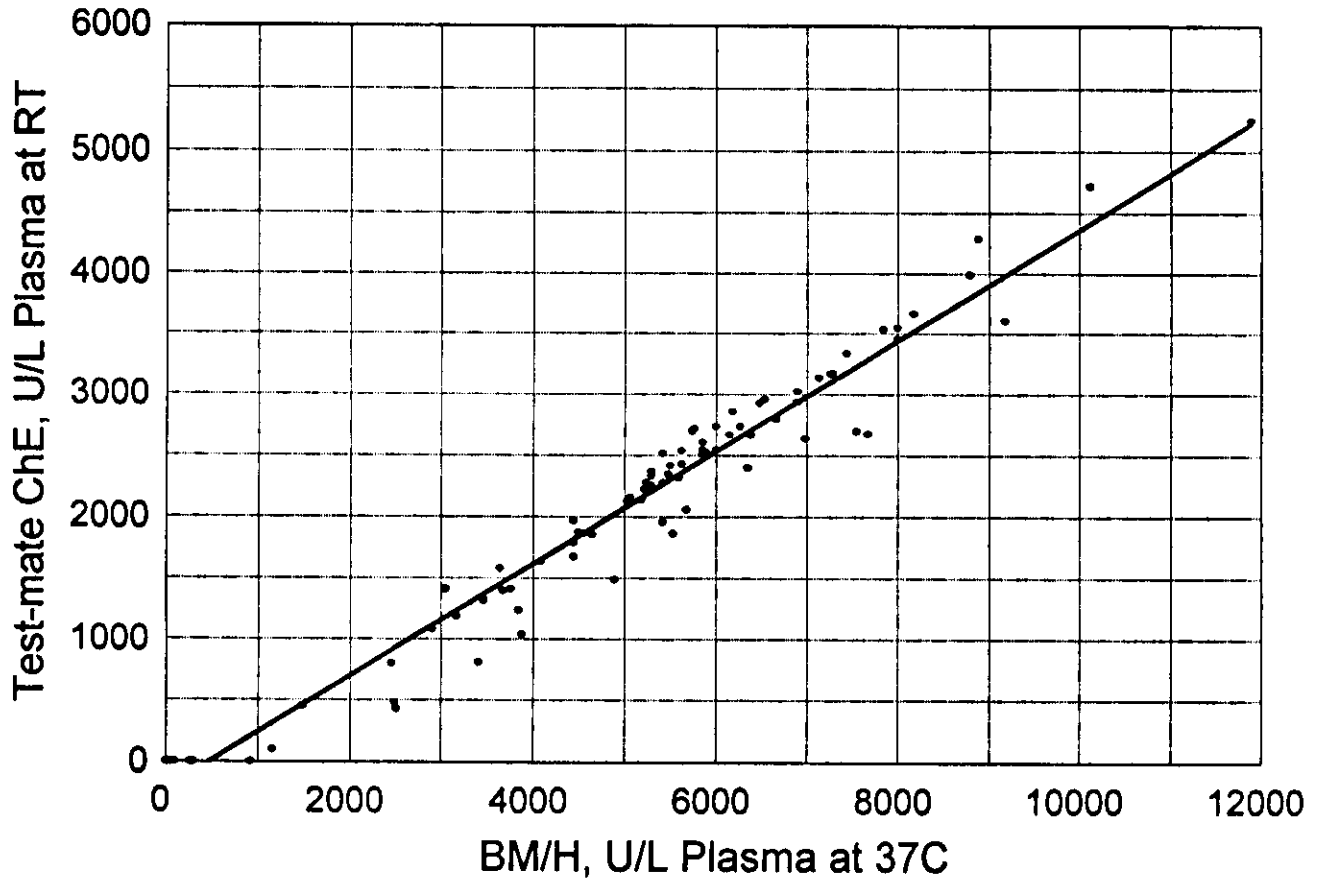
Regression Output:

Constant	439.9503
Std Err of Y Est	126.5664
R Squared	0.91697
No. of Observations	44
Degrees of Freedom	42
X Coefficient	0.252523
Std Err of Coef.	0.011725
Correlation Coef.	0.957586

Fig. 14-C

PChE: Same Sample Analysis

Including Pesticide Spiked Samples



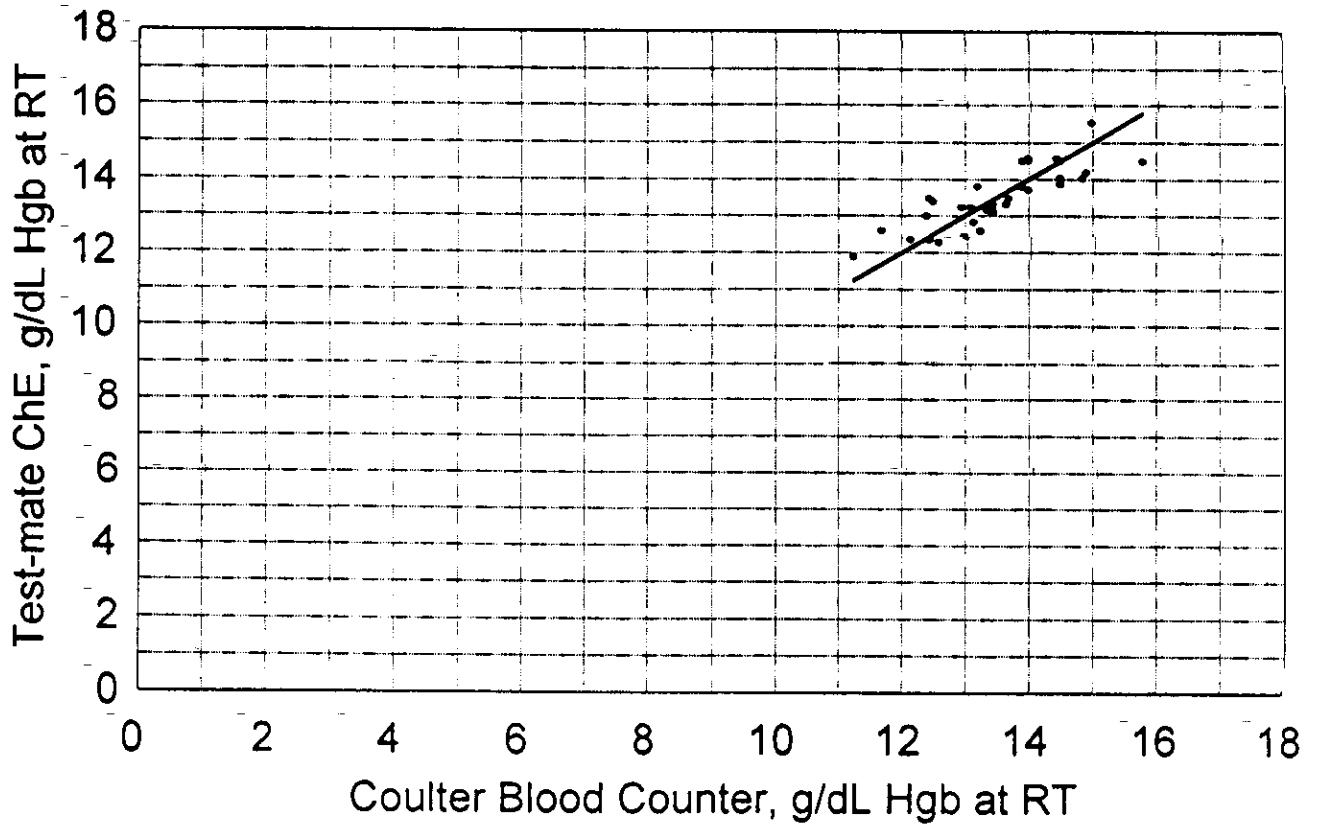
Regression Output:

Constant	-210.405
Std Err of Y Est	226.6717
R Squared	0.963932
No. of Observations	87
Degrees of Freedom	85
X Coefficient	0.457076
Std Err of Coef.	0.00959
Correlation Coef.	0.981800

Fig. 14-D

Hgb: Normal Population Analysis

Venipuncture vs. Fingerstick, respectively



Regression Output:

Constant	2.266081
Std Err of Y Est	0.599665
R Squared	0.69022
No. of Observations	33
Degrees of Freedom	31
X Coefficient	0.871199
Std Err of Coef.	0.104826
Correlation Coef.	0.830795

Fig. 14-E