Procedural Section

1.0 Scope and Application

1.1 The Hitachi 911 Automatic Analyzer (Boehringer Mannheim, Indianapolis, IN) is used to run a wide range of chemistries, e.g., cholinesterase.

1.2 The Hitachi 911 is a spectrophotometer equipped with sophisticated robotics that automatically dispense samples and appropriate reagents into cuvettes suspended in a 37°C water bath. Absorbance readings are taken and enzyme activity is calculated by the analyzer.

1.3 These analyzers are used extensively in many testing and clinical laboratories to ensure reproducibility and increase sample throughput. However, these instruments are not appropriate to use in the determination of cholinesterase activity in carbamate-treated tissues, e.g., carbaryl, due to reactivation of cholinesterase activity that occurs during the analyzer's performance of the assay.

2.0 Prerequisites

2.1 Equipment and Supplies

Hitachi 911 Automatic Analyzer*
Sample Cups*
Reagent Bottles*
Pipet, 200 µl
Laboratory Glassware
Analytical Balance
Ice

*Analyzer and its supplies obtained from Boehringer Mannheim, Indianapois, IN

All other equipment and supplies are considered standard laboratory devices or items and do not need to meet critical specifications.

2.2 Chemicals

Acetylthiocholine Iodide (Sigma, A-5751)
5,5'-dithio-bis(2-nitrobenzoic) Acid (DTNB) (Sigma, D-8130)
0.2M Sodium Phosphate, monobasic (Sigma, S-9638): 27.6 g NaH_2PO_4 in1000 ml ddH2O
0.2M Sodium Phosphate, dibasic (Sigma, S-9763): 28.4 g Na_2HPO_4 in 1000 ml ddH2O
0.1M Sodium Phosphate Buffer, pH 8.0: 380 ml 0.2M Na_2HPO_4 (dibasic) + 20 ml 0.2M

NaH2PO4 (monobasic), adjust pH to 8.0, double volume by adding ddH2O and refrigerate

0.1M Sodium Phosphate Buffer, pH 8.0 + 1% Triton (Sigma, T-6878)

0.1M Sodium Phosphate Buffer, pH 7.0: 61 ml 0.2M Na2HPO4 (dibasic) + 39 ml 0.2M NaH2PO4 (monobasic), adjust pH to 7.0, double volume by adding ddH2O and refrigerate

DTNB Stock Solution: 118.8 mg DTNB
45.0 mg Sodium Bicarbonate (Sigma, S-233)
30.0 ml 0.1M Sodium Phosphate Buffer, pH 7.0
Freeze

Saline- Laboratory grade

2.3 Biological

Samples should be stored at -80°C in an alarm equipped freezer

Tissues will be received already appropriately diluted and homogenized in 0.1M Sodium Phoshate Buffer, pH 8.0 containing 1% Triton

Keep samples on ice during preparation

2.4 Personnel Training

Personnel must complete the manufacturer's 8-day Hitachi 911 Basic Operator Course at the Boehringer Mannheim Training Center, Indianapolis, IN to become knowledgable in the operation of the automatic analyzer.

3.0 Special Considerations

3.1 Because tissues need to be more dilute to accomodate the robotics of the analyzer and the analyzer employs a manufacturer's set preincubation period, tissues that have been treated with a carbamate pesticide should not be assayed using an automatic analyzer. These situations lead to reactivation of cholinesterase activity and thus, results will not be accurate.

4.0 Procedure

1. Tissues may require additional dilution using 0.1M Sodium Phosphate Buffer, pH 8.0 containing 1% Triton to be run on the analyzer. Based on historical data generated in the laboratory using the automatic analyzer, tissues should be diluted as follows:
   Plasma- undiluted
   Erythrocytes- (1:25) dilution
   Brain- (1:50) dilution
   Heart- (1:50) dilution
2. Prepare substrate as follows:
   a. 2.444 mg Acetylthiocholine Iodide + 1.0 ml ddH2O

b. Each cholinesterase test requires 50 µl of substrate
c. Prepare the proper amount of substrate according to the following formula:
X*2=Y
Y*50 µl=Z
Where: X= single # of samples to be run
Y= total # of samples
Z= ml of substrate required
d. Weigh out acetylthiocholine iodide and divide by 2.444 to determine the volume of ddH2O to be added.
Example: 32 samples are to be run.
32* 2=64
64* 50=3.2 ml of substrate required
26.4 mg of acetylthiocholine iodide is weighed out
26.4 mg/2.444= 10.8 ml of ddH2O added

3. Similarly prepare the DTNB/Buffer solution according to the following:
a. 1 ml DTNB (from frozen stock) + 29 ml 0.1M Sodium Phosphate, pH 8.0 Buffer
b. Each cholinesterase test and its blank requires 300 µl of DTNB/Buffer
c. Prepare the proper volume of DTNB/Buffer according to the following formula:
X* 4=Y
Y* 300 µl=Z
Where: X= single # of samples to be run
Y= total # of samples
Z= ml of DTNB/Buffer required
Example: Same 32 samples to be run.
32* 4=128
128* 300=38.4 ml DTNB/Buffer required
3 ml DTNB + 87 ml 0.1M Sodium Phosphate Buffer, pH 8.0
4. Pour substrate into a barcoded reagent bottle. Split the volume of DTNB/Buffer into reagent bottles. Fill a third reagent bottle with saline to be run as a blank. Place reagent bottles into their assigned positions on the reagent disk.
5. Perform a calibration of the analyzer and run controls
6. Pipet 200 µl of sample into sample cup and place it on sample disk
7. When sample disk is filled, place on analyzer and program analyzer
8. Analyzer will calculate cholinesterase activity and print results

Quality Control Section

1.0 All samples are run in duplicate.

1.1 The analyzer uses the analysis of blanks.

1.2 The analyzer operator runs a set of brains at the following dilutions: (1:50), (1:100), (1:200) and (1:400) to be used as controls to verify that reagents were prepared correctly and the machine is operating properly prior to any samples being run. Control values are archived bythe analyzer and are checked in the QC Parameter function to verify that the values reported for each activity remain in a tight range (±50 units).

1.3 A calibration of the analyzer is performed each day that the machine is used.

1.4 Calculations:

a. The analyzer reports results in an undefined unit/liter.

b. Since this measurement is not significant in the science field, all data collected from the analyzer is expressed as % of control.

c. To ensure that the data generated from the automatic analyzer is accurate, a subset of samples are run on both the analyzer and by the radiometric method. When the data from both methods are calculated as % of control, the percentages are within ±5%.

1.5 Recordkeeping Requirements

All sample inventories, correspondance, etc. for a study are kept in a labeled hanging file folder in the laboratory's file cabinet. As assays are run, notations on procedure, including any problems, are documented in the technician's laboratory notebook. Resulting data is organized in Excel (Microsoft Office '97) files. Hardcopies are printed and placed in the study file. The printouts generated by the automatic analyzer are labeled at the top with the study name, tissue assayed, tissue dilution and assay date before being placed in the study file.