The Influence of Blood Sample Storage Conditions on Cholinesterase Activity
Introducing a Portable Cholinesterase Test Kit into Clinical Practice

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Specific Aims

Overall Goal: To introduce a portable, clinician-based cholinesterase (ChE) testing methodology into practice as a means of monitoring ChE in agricultural workers in Washington State.

- To investigate the effect of storage environments we will analyze ChE activity of blood stored in four different environments likely to be encountered in the field and clinical setting:
  - Freezer (-20°C), refrigerator, ice-cooler, and at room temperature.
- To further explore the possibilities of storing blood samples we will test what effect of storing blood preserved in buffer solution (used in Test-mate analysis).

What is cholinesterase?

- ChE is an enzyme found in humans, insects, and other invertebrates, that is critical for the proper function of the nervous system.
- Acetylcholine (ACh) is a key neurotransmitter which signals nerve impulses between the junctions of nerves and muscles, glands, and other nerves in the central and peripheral nervous systems (Fig. 1).
- ChE (acetylcholinesterase) breaks down ACh into acetate and choline, returning the stimulating signal and allowing the nerve to return to an "off" condition.
- There are two types of ChE:
  - Acetylcholinesterase (AChE, "true" cholinesterase, erythrocyte cholinesterase)—found primarily in blood and neural synapses; prefers substrate is acetylcholine.
  - Butyrylcholinesterase (pChE, BuChE, pseudocholinesterase, plasma cholinesterase)—found in plasma, prefers substrate butyrylcholine and/or propionylcholine. No known essential function.

Cholinesterase Monitoring in Washington State

In 2002 the Washington State Department of Labor & Industries initiated a monitoring program to prevent pesticide-related illnesses among agricultural workers who handle ChE-inhibiting pesticides.

- The program monitors those workers who handle OP and CB pesticides for more than 30 hours in a given 30-day period.
- Participating workers are tested prior to exposure to obtain baseline AChE and pChE activity measurements for comparison. Follow-up ChE measurements are then taken at least once every 30 days if the 30-hour threshold is exceeded.
- If a worker experiences a 20% reduction from baseline for either AChE or pChE, the employer must investigate possible causes of overexposure.
- Workers who experience more than a 30% reduction in AChE or more than a 40% reduction in pChE must be removed from handling activities until their ChE activity levels return to within 20% of baseline.

Toxicity of Pesticides - ChE Inhibitors

- Two types of pesticides, organophosphates (OPs) and N-methyl carbamates (CMs), both of which are widely used in agriculture are toxic because of their inhibition of ChE.
- Inhibition of ChE can lead to an accumulation of ACh at the synaptic cleft, leading to overstimulation of the post-synaptic tissue.
- Symptoms of ChE depression include:
  - Mild cases: tiredness, weakness, dizziness, nausea, and blurred vision.
  - Moderate cases: headache, sweating, trembling, drowsiness, vomiting, tunnel vision, and twitching.
  - Severe case: abdominal cramps, aching, diarrhea, muscular tremors, staggering gait, hypotension, slow heartbeat, breathing difficulty, and possibly death.

Why Use a Portable ChE Test Kit?

- Current methods of ChE monitoring in Washington State require that samples be shipped from clinic where blood is drawn to a laboratory for testing.
- Lag-time between sample collection and analysis puts workers with depressed ChE levels in greater risk of further exposure.
- On-site ChE testing at medical clinics would allow for faster results and more effective protection of exposed pesticide handlers.
- Additionally, implementation of on-site clinical testing of ChE would eliminate problems associated with shipment such as improper temperature control, leaking of samples, and homologization.

The EOM-testmate™ ChE System

- The test-mate is a photometric analyzer that measures ChE activity based on the Ellman method.
- AChE/buthyrylcholinesterase (BuChE) is hydrolyzed by AChE or pChE, respectively, producing thiocholine and 5-thiol-2-nitrobenzoic acid which reacts with the Ellman reagent (DTNB) to produce a yellow color.
- The rate of the formation of the yellow color is proportional to the amount of either AChE or pChE and is measured spectrophotometrically at 450nm.
- To date, researches have used the Test-mate kit to measure ChE activity in both the field and laboratory settings.
- While reports on the kit’s validity and reliability have generally supported the technology, some concern over the stability of measurements in environments with changing temperature or below 20°C.

Stability of Cholinesterase

- Due to the relatively short half-life of ChE, all baseline blood must be measured for subsequent comparison, although not all workers return for follow-up measurements.
- Storing blood for extended period of time would allow simultaneous comparison with a baseline sample for only returning individuals, thereby making monitoring ChE more cost-effective.
- Whitt reported that pChE activity was maintained for several weeks after stored at 4°C.
- Hsia et al reported no change in ChE activity in muscle samples stored at room temperature, in a refrigerator, and in a freezer for up to 50, 90 days, and 52 weeks, respectively.
- Similar results were achieved in an analysis of bovine blood samples that showed stable ChE activity of samples stored at 37°C for two days.
- All mentioned studies analyzed ChE activity in a controlled laboratory setting. What is lacking in current knowledge is what effect storage of blood samples in storage environments likely available and utilized in a clinical setting has on ChE activity as measured by a portable test kit.

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