

TECHNICAL BULLETIN

**OCCUPATIONAL AND ENVIRONMENTAL
HEALTH**

**RED BLOOD CELL-CHOLINESTERASE TESTING
AND QUALITY ASSURANCE**

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HEADQUARTERS, DEPARTMENT OF THE ARMY
30 NOVEMBER 2001

RED BLOOD CELL-CHOLINESTERASE TESTING AND QUALITY ASSURANCE

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*This bulletin supersedes TB MED 292, 30 May 1975.

CHAPTER 1

INTRODUCTION

1-1. Purpose

This bulletin—

a. Describes the methodology for measuring red blood cell cholinesterase (RBC-ChE) activity in the Department of Defense (DOD) Cholinesterase Monitoring Program.

b. Provides information to—

(1) Installation commanders who have a ChE-inhibiting substance mission.

(2) Health care providers who are responsible for evaluating RBC-ChE depressions and for maintaining the RBC-ChE test records. (See DA Pam 40-8.)

(3) Laboratory personnel responsible for conducting RBC-ChE assays.

c. Describes the controls and standards included in the DOD laboratory test method.

d. Describes the procedures for—

(1) Requesting RBC-ChE assay analysis.

(2) Collecting and processing blood specimens.

(3) Completing test results.

(4) Reporting test results.

e. Describes the procedures used by the Cholinesterase Reference Laboratory (CRL) at the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) to—

(1) Support standardized testing requirements.

(2) Provide limited primary testing.

(3) Establish and maintain quality control (QC) and quality assurance (QA) procedures.

(4) Maintain assay performance standardization by—

(*a*) Specifying procedures, reagents, and instrumentation.

(*b*) Providing technical training and consultation.

(*c*) Certifying laboratory technicians.

(5) Maintain transferability of test results throughout the program by performing—

(*a*) Test result validation by representative specimen retesting.

(*b*) Statistical analysis of test-retest differences.

(6) Conduct onsite laboratory audits at least annually.

1-2. References

Appendix A lists the references used in this bulletin.

1-3. Abbreviations and terms

The glossary provides a list of abbreviations and terms used in this bulletin.

1-4. Background

a. Cholinesterase testing is an essential element in the medical surveillance of chemical agent and pesticide workers. The enzyme ChE hydrolyzes acetylcholine resulting in the release of acetic acid and choline. This mechanism is important for the breakdown of the neurotransmitter acetylcholine to retain normal function of nervous, muscle, and gland tissues. Acetylcholine inhibiting compounds, such as the nerve agents GA, GB, GD, VX and organophosphorous insecticides, bind to ChE, rendering the enzyme inactive. This inhibition will result in concentration dependent compromise of nervous system function, which can eventually lead to death.

b. The RBC-ChE is a representative of the true ChE of the nervous system. Exposure to ChE-inhibiting compounds, even at sub-clinical levels, can be detected by monitoring RBC-ChE activity. Exposure to inhibitors is detected by comparing an individual's RBC-ChE activity level to a previously established baseline level and checking for a depression in enzyme activity.

c. The DOD has adopted the use of a single standardized method for RBC-ChE testing: the Manual 17-Minute Change in pH Method. A QA program has been established to ensure—

(1) The accuracy and precision of the measurements.

(2) That the results obtained by all testing sites are comparable.

1-5. Functions

a. The USACHPPM CRL—

(1) Performs RBC-ChE QA oversight for nerve agent stockpile and demilitarization sites.

(2) Provides limited primary RBC-ChE testing services to DOD organizations and DOD contractors.

(3) Evaluates and approves testing site laboratories' requests for RBC-ChE testing.

(4) Provides training to testing site laboratory staff.

(5) Provides select testing equipment to testing sites on a loan basis.

(6) Provides standing operating procedures (SOPs) pertinent to RBC-ChE testing.

b. RBC-ChE testing sites—

(1) Request admission to the QA program from the CRL.

(2) Provide qualified technicians to the CRL for training.

(3) Dedicate adequate testing facilities and supplies.

(4) Maintain test equipment on loan from the CRL.

(5) Submit RBC-ChE specimens with appropriate patient information to the CRL in a timely manner.

c. Primary sites submit RBC-ChE specimens with appropriate patient information to the CRL in a timely manner.

1-6. Technical assistance

To contact the CRL on safety or other issues, write to Commander, U.S. Army Center for Health Promotion and Preventive Medicine, 5158 Blackhawk Road, ATTN: MCHB-TS-LRD, Aberdeen Proving Ground, MD 21010-5403.

CHAPTER 2

RBC-ChE QUALITY ASSURANCE PROGRAM

2-1. Authority

DA Pam 40-8 authorizes the QA program for RBC-ChE testing at nerve agent stockpile and demilitarization.

2-2. Background

To ensure a consistent and reliable ChE testing environment within DOD, the CRL provides QA services and oversight for the RBC-ChE testing program. This program verifies performance for each testing site.

2-3. Program participation

Eligible laboratories engaged in RBC-ChE testing within the DOD RBC-ChE surveillance mission, must participate in the QA program. To join the RBC-ChE testing program, testing site laboratories should contact the CRL. The following requirements must be fulfilled before new laboratories can be accepted as RBC-ChE testing sites.

a. Laboratory personnel must meet the standards for high complexity testing set forth by the DOD Clinical Laboratory Improvement Program (DOD CLIP) in the Armed Forces Institute of Pathology (AFIP) Pamphlet 40-24.

b. Laboratories performing RBC-ChE testing must be CLIP or Clinical Laboratory Improvement Amendments (CLIA) registered and accredited by either Commission on Office Laboratory Accreditation (COLA), College of American Pathologists (CAP), or Joint Commission on Accreditation of Healthcare Organizations (JCAHO).

c. New laboratories should submit a written justification to USACHPPM CRL explaining the expected duration of RBC-ChE testing needed and an approximate number of blood specimens expected to be processed.

d. The CRL will assign an account number to each laboratory that participates in the DOD RBC-ChE monitoring program. This account number must accompany each specimen submitted to the CRL to ensure proper processing of specimens. The program manager for the submitting laboratory will be responsible for providing the CRL with the account information. Reimbursement for CRL services will be determined on an individual basis.

However, services provided to military laboratories are generally reimbursable if not covered by central funding.

2-4. CRL services

The CRL will provide the following services:

a. *Training and certification in the Manual 17-Minute Change in pH Method.* The initial technician training consists of a 40-hour training block at the CRL, which includes hands-on experience in ChE determination, operation and maintenance of ChE testing equipment, and QC procedures. Upon successful completion of this training, technicians will receive a certificate. Annual technician recertification will be given based on ChE testing performance during the previous year and performance assessed during site visits.

b. *Quarterly proficiency surveys of participating laboratories.* Blind specimens will be sent to testing sites on a quarterly basis. Results from all the participating laboratories will be compiled, and individual sites must stay within ± 2 standard deviations of the population mean.

c. *RBC-ChE testing performance monitoring.*

(1) After completion of onsite testing, the testing sites will send all specimens to the CRL. To ensure continued satisfactory testing performance, 20 to 30 percent of specimens from testing sites will be retested at the CRL. The results from testing sites must fall within ± 0.05 pH units, when compared to the CRL results obtained on the same specimen, to meet acceptability criteria.

(2) The CRL will provide the testing site with a written report of the comparative test. If results do not meet acceptability criteria, the CRL staff will remotely aid (that is, telephone and or email) the testing sites in troubleshooting. If remote troubleshooting does not solve the problem, the CRL will conduct an onsite visit. The expenses for this visit are the responsibility of the testing site.

d. *Onsite laboratory audits.* The CRL will conduct annual audits to assure testing site operations are in compliance with DOD RBC-ChE program standards. If laboratories show poor performance, the CRL may conduct audits more frequently. The CRL will furnish the laboratories an assessment report summarizing any deficiencies

found during the audits. Laboratories must correct the deficiencies and submit a written report of corrective actions taken to the CRL within 60 days after the audit.

e. QC specimens. The CRL will provide QC. Testing sites must keep QC charts for control results, and all corrective actions taken for controls that do not meet acceptability criteria will be logged on QC charts.

f. Calibration of equipment. All equipment used for quantitative analysis or the purpose of collecting quantitative data must be calibrated in accordance with CRL or local SOPs. If available, National Institute of Standards and Testing (NIST) traceable reference materials must be used for calibration.

2-5. Requesting RBC-ChE analysis

Blood specimens submitted for RBC-ChE testing should be accompanied by a request memorandum containing the following information:

- a.* Patient name.
- b.* Age.
- c.* SSN.
- d.* Type of test ordered.
- e.* Name and signature of physician ordering the test.
- f.* Date test ordered.

- g.* Patient's baseline (if available) and category.
- h.* Date and time specimen was taken.
- i.* Address where test results are to be reported.

2-6. Submitting specimens for QA analysis to CRL

Specimens will be submitted to the CRL per USACHPPM SOP CRL 40-10.

2-7. Reporting test results

To ensure effective ChE health monitoring, testing laboratories will—

a. Provide a written report of the results for primary RBC-ChE determinations within 72 hours of specimen receipt for category A personnel and within 1 week of specimen receipt for all other categories (B, C, and D).

b. Inform the referring health care provider or installation medical authority (IMA) immediately if RBC-ChE depressions equal to or greater than 25 percent of baseline are detected.

c. Refer variations of greater than 10 percent from baseline value (low or high) to the IMA for further analysis.

2-8. Program procedures

Detailed procedures for the QA program may be found in USACHPPM SOPs CRL 40-9, 40-13, and 40-15.

CHAPTER 3

PRIMARY ChE TESTING

3-1. Authority

DA Pam 40-8 authorizes limited primary ChE testing for DOD agencies and contractors.

3-2. Background

To provide ChE testing capability for Government sites and Government contractors without onsite testing facilities, the CRL performs ChE determinations for specimens submitted to the CRL on a limited basis.

3-3. Program participation

a. New customers need to provide the CRL with the following information:

(1) Written justification of the service needed and the expected duration of this service.

(2) A point of contact for administrative issues.

(3) A complete mailing address for test results.

b. The CRL will assign an account number to all customers participating in the primary testing program. This account number must accompany all primary specimens submitted to the CRL to ensure proper processing of specimens. The program manager for the submitting site will be responsible

for providing the CRL with the account information. In general, services provided to civilian contractors are reimbursable.

3-4. Submitting primary specimens to CRL

Specimens should be submitted to the CRL per USACHPPM SOP CRL 40-10. Specimens must be submitted with the following information:

a. Patient name.

b. Age.

c. SSN.

d. Type of test ordered.

e. Name and signature of physician ordering the test.

f. Date test ordered.

g. Patient's baseline (if available) and category.

h. Date and time specimen was taken.

i. Address where test results are to be reported.

3-5. Reporting test results

The CRL will furnish a written report with the ChE test results to the submitting site within 3 days of receipt of specimen.

CHAPTER 4

PROCEDURES FOR PERFORMING RBC-ChE TESTING

4-1. Purpose

This chapter provides and explains the procedures for—

- a. Collecting and processing blood specimens.
- b. Preparing reagents.
- c. Analyzing blood specimens.
- d. Using equipment and materials in the manual method.
- e. Using the reference range.
- f. Determining the initial RBC-ChE activity baseline value.

4-2. Safety and administrative procedures

Follow all standard laboratory safety and administrative procedures, to include—

- a. SOPs recommended by the CAP, COLA, and CLIP/CLIA.
- b. Local bloodborne pathogens exposure control SOP.
- c. Local chemical hygiene plans.
- d. Local safety SOPs.
- e. Part 1910.1030, title 29, Code of Federal Regulations (CFR), 1991.
- f. Volume 56, Federal Register, p. 235.
- g. Part 1910.1200, title 29, Code of Federal Regulations.

4-3. Blood specimen collection

- a. Collect blood specimens per USACHPPM SOP CRL 40-10 and local SOPs on phlebotomy.
- b. Centrifuge the specimen to separate the RBCs from the plasma and remove the plasma within 4 hours of collection.

4-4. Reagent preparation

a. All reagents will be prepared per USACHPPM SOP CRL 40-2. All chemicals and solutions must be labeled with dates received or prepared, date opened and expiration date, and must be stored properly.

b. The following solutions are required for RBC-ChE testing:

- (1) Acetylcholine bromide solution for enzyme substrate, 0.11 moles per liter (M).
- (2) Sodium barbital (0.01 M) -phosphate (0.002 M) stock buffer, pH 8.19-8.25.

(3) Sodium barbital (0.01 M) -phosphate (0.002 M) -saponin (0.015 % weight per volume) working buffer, pH 8.05-8.10.

4-5. Blood specimen analysis

a. The RBC-ChE assay currently in use is based on a modification (R.I. Ellin, et al., 1973) of a potentiometric assay method developed by H.O. Michel (1949). The enzyme activity is expressed in units of change in pH per hour (Δ pH/h).

b. To set up the standard equipment and perform the enzyme assay—

(1) Prepare the water bath, pH meter, and dual syringe diluter per USACHPPM SOPs CRL 40-5, CRL 40-6, and CRL 40-3.

(2) Perform a balance verification on the analytical balance. Refer to USACHPPM SOP CRL 40-9 and the operator's manual for this procedure.

(3) Equilibrate the blood specimens and reagents. Allow all specimens, reagents, and controls to equilibrate to room temperature.

(4) Prepare the working buffer to be used in this method.

(5) Prepare the RBC-ChE data runsheet.

(6) Prepare specimens per USACHPPM SOP CRL 40-2.

(7) Perform the enzyme assay per USACHPPM SOP CRL 40-2.

(8) Calculate the RBC-ChE activity in units of Δ pH/h per USACHPPM SOP CRL 40-2.

4-6. Instruments and equipment required to perform RBC-ChE assays

To ensure accurate results and comparability of results between laboratories, equipment for RBC-ChE testing must be standardized.

a. The CRL, on a loan basis, will provide the following equipment:

- (1) pH meter.
- (2) Dual syringe diluter.
- (3) Constant temperature water bath.

b. This equipment will remain the property of the CRL and will not be transferred to any testing facility property book. The equipment will be temporarily loaned to testing sites on DA Form 2062 (Hand Receipt/Annex Number).

c. Each testing site will receive two sets of the equipment items mentioned in paragraph a above. One equipment item will be in use and the other

will be maintained as a back up. In the event of equipment failure, the testing facility must coordinate with the CRL promptly to arrange for repair or replacement. Only user maintenance is authorized. All other repairs must be coordinated and approved by the CRL.

d. Costs for the repair and replacement of equipment will be paid by the CRL. Shipping costs are the responsibility of the testing facility.

e. The CRL will provide operator's manuals and SOPs for each item.

f. A list of recommended equipment and consumables is available from the CRL. Each lab-

oratory is responsible for purchasing and maintaining these items.

4-7. Reference range

The reference range for RBC-ChE activities is 0.63–0.89 Δ pH/h. This reference range should only be used in an emergency in the absence of patient baseline values.

4-8. Baseline determination

RBC-ChE baseline levels will be determined per DA Pam 40-8.

APPENDIX A

REFERENCES

A-1. Department of Defense Publications

AFIP Pamphlet 40-24, Technical Instructions for the Department of Defense Clinical Laboratory Improvement Program. (Available from Armed Forces Institute of Pathology, 6825 16th Street NW, ATTN: CLIP Office, Washington, DC 20306-6000.)

A-2. Department of the Army Publications

DA Pam 40-8, Occupational Health Guidelines for the Evaluation and Control of Occupational Exposure to Nerve Agents GA, GB, GD, and VX

A-3. Cholinesterase Reference Laboratory Standing Operating Procedures (Available from USACHPPM, 5158 Blackhawk Road, Aberdeen Proving Ground, MD 21010-5403)

CRL 40-2, 17-Minute RBC Acetylcholinesterase Procedure
CRL 40-3, Operation of the Dual Syringe Diluter
CRL 40-4, Calibration of the Substrate Repipet
CRL 40-5, Operation of Precision Circulating Water Bath
CRL 40-6, Operation of Models 345 and 445 pH Meters
CRL 40-8, Operation of the Virtis Unitop Model 600SL Freeze Dryer
CRL 40-9, Quality Assurance Program
CRL 40-10, Sample Collection, Processing, Shipping and Receiving
CRL 40-11, Bloodborne Pathogens Exposure Control
CRL 40-13, Preparation of QA/QC Samples
CRL 40-14, Data Entry
CRL 40-15, Proficiency Testing

A-4. Other publications

Ellin R.I., Burkhardt B.H., and Hart R.D. (1973), A Time-Modified Method for Measuring Red Blood Cell Cholinesterase Activity. *Arch. Environ. Health* 27:48-49
Michel, H.O. (1949), An Electrometric Method for the Determination of Red Blood Cell and Plasma Cholinesterase Activity. *J. Lab. Clinic. Med.* 34: 1564-1568
29 CFR 1910, Occupational Safety and Health Standards

A-5. Referenced forms

DA Form 2062
Hand Receipt/Annex Number

GLOSSARY

Section I. ABBREVIATIONS

AFIP

Armed Forces Institute of Pathology

CAP

College of American Pathologists

CAS

Chemical Abstract Service

CFR

Code of Federal Regulations

ChE

cholinesterase

CLIA

Clinical Laboratory Improvement Amendments

CLIP

Clinical Laboratory Improvement Program

COLA

Commission on Office Laboratory Accreditation

CRL

Cholinesterase Reference Laboratory

DOD

Department of Defense

FR

Federal Register

IMA

installation medical authority

JCAHO

Joint Commission on Accreditation of Healthcare Organizations

M

moles per liter

MTF

Military Treatment Facility

TB MED 590

NIST

National Institute of Standards and Testing

Δ pH/h

change in pH per hour

QA

quality assurance

QC

quality control

RBC

red blood cell

SF

standard form

SOP

standing operating procedure

USACHPPM

U.S. Army Center for Health Promotion and Preventive Medicine

Section II. TERMS

Agent GA

The chemical dimethylphosphoramido-cyanidate, Chemical Abstracts Service (CAS) registry number 77-81-6, in pure form and in the various impure forms which may be found in storage as well as in industrial, depot, or laboratory operations.

Agent GB

The chemical isopropyl methylphosphono-fluoridate, CAS registry number 107-44-8, in pure form and in the various impure forms which may be found in storage as well as in industrial, depot, or laboratory operations.

Agent GD

The chemical phosphonofluoridic acid, methyl-1, 2, 2-trimethylpropyl ester, CAS registry number 96-64-0, in pure form and in the various impure forms which may be found in storage as well as in industrial, depot, or laboratory operations.

Agent VX

The chemical phosphonothioic acid, methyl-, S- (2- (bis (1-methylethyl) amino) ethyl) 0-ethyl ester, CAS registry number 50782-69-9, in pure form and in the various impure forms which may be found in storage as well as in industrial, depot, or laboratory operations.

Category A personnel

Personnel with a high risk of potential exposure due to the nature of the agent operations being conducted. (See DA Pam 40-8, para 4-2.)

Category B personnel

Personnel with a low risk or infrequent potential exposure to nerve agents in routine industrial, laboratory, or security operations and job requirements involving the prolonged wearing of protective ensembles during training and emergency responses. (See DA Pam 40-8, para 4-2.)

Glossary-2

Category C personnel

Personnel with minimal probability of exposure to nerve agents even under accident conditions, but whose activities may place them in close proximity to agent areas. (See DA Pam 40-8, para 4-2.)

Category D personnel

Transient visitors to agent areas where there is a potential for exposure and who are not included in the medical surveillance program for nerve agents at the visited installation. (See DA Pam 40-8, para 4-2.)

Data runsheet

Worksheet that lists all the specimens analyzed during a single assay run that is used for entering raw data and computing RBC-ChE results.

Primary site

Any site submitting an RBC-ChE specimen to the CRL for determination of RBC-ChE enzyme activity and which does not have onsite RBC-ChE testing capability.

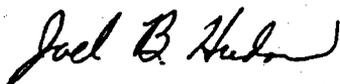
Testing site

Any RBC-ChE testing location that is currently certified by the RBC-ChE QA program and is participating in the RBC-ChE QA program.

By Order of the Secretary of the Army:

ERIC K. SHINSEKI
General, United States Army
Chief of Staff

Official:



JOEL B. HUDSON
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Distribution:

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