CLIA-Compliant Analytical Method Validation Plan and Template

FOR LRN-C LABORATORIES

TABLE OF CONTENTS

LIST OF ACRONYMS

EQA	External Quality Assessment
HPLC-MS/MS	High-Performance Liquid Chromatography Tandem Mass Spectrometry
IS	Internal Standard
LDT	Laboratory Developed Test(s)
LOD	Limit of Detection
LOQ	Limit of Quantitation
PK/PD	Pharmacokinetic/Pharmacodynamic modeling
TE_a	Total Allowable Error
ULOL	Upper Limit of Linearity

INTRODUCTION

The Laboratory Response Network[i] (LRN) and its partners maintain an integrated national and international network of laboratories that can respond quickly to acts of chemical, radiological or biological terrorism, emerging infectious diseases and other public health threats and emergencies.[1] The Laboratory Response Network for Chemical Threats[ii] (LRN-C), representing over 40 public health laboratories, remains dedicated to the establishment and preparedness of analytical toxicology services that meet needs for emergency response, investigation and patient care.

The Clinical Laboratory Improvement Amendments[iii] (CLIA) applies to LRN-C clinical tests (tests on human blood, urine or other tissue) as do LRN Network requirements. These dual requirements include method validation procedures. The Association of Public Health Laboratories[iv] assembled a Taskforce tasked with providing a guidance document to assist LRN-C laboratories in meeting these requirements.

This document is provided for general guidance purposes only. Individual laboratories and laboratory directors remain responsible for meeting all CLIA'88 clinical laboratory regulations (42 CFR 493)[2] and may have specific needs not addressed by this document. Modifications may be required to meet all applicable federal regulations.

LRN CLIENTS AND RESPONSIBILITIES

An understanding of the needs and expectations of potential clients in emergency preparedness and response is essential to establishing minimum requirements for CLIA-compliant performance specifications, policies and procedures.

With regard to potential clients, the LRN-C provides analytical and investigative support to public health officials, epidemiologists, toxicologists, healthcare providers, law enforcement, environmental health and food safety specialists, and poison control centers. LRN-C helps determine the cause and origin of a public health incident and to definitively characterize the organism or agent responsible. National Standards for Public Health Preparedness at the State and Local Level[v] include the ability to perform the following:

Function 1: Manage laboratory activities
Function 2: Perform sample management
Function 3: Conduct testing and analysis for routine and surge capacity
Function 4: Support public health investigations
Function 5: Report results

QUALITY REQUIREMENTS FOR ANALYTICAL SERVICES

A quality management system must be in place to ensure the delivery of timely and reliable services. The recommendations put forth in this document address only the establishment of performance specifications for analytical methods and the validation of method performance characteristics. The laboratory is responsible for good laboratory practices and compliance with

---

1 APHL. *Laboratory Response Network.* http://www.aphl.org/aphlprograms/preparedness-and-response/laboratory-response-network/pages/default.aspx, *accessed May 2013.*

2 Centers for Medicare and Medicaid Services, HHS. Standards and Certifications: Laboratory Requirements. Part 493. http://wwwn.cdc.gov/clia/Regulatory/default.aspx.

regulatory program requirements for all phases of laboratory testing.

The LRN-C uses laboratory-developed tests (LDTs) to analyze clinical specimens for toxic substances identified as possessing high risk and consequence to the public health. CLIA'88 rules require the laboratory to establish performance specifications and to validate method performance for the following method characteristics:

- accuracy
- precision (within run, between run, between day)
- reportable range
- analytical sensitivity and specificity
- reference intervals

CLIA rules and guidance[3] are silent on minimum requirements for validation protocols; however, validation plans, experiments, results and conclusions are rigorously reviewed for scientific merit and integrity. The laboratory director is responsible for establishing individual laboratory criteria. The validation plan and template provided in this document:

- guides the laboratory director in the establishment of method performance specifications considering the intended use of the analytical findings
- meets or exceeds minimum regulatory and accreditation program requirements for LDT validation

ANALYTICAL METHOD PERFORMANCE SPECIFICATIONS

A. Accuracy and Precision[4],[5]

The following is a hierarchy of recommended approaches to the establishment of requirements for the accuracy and precision of clinical analytical methods.

1. **Medical usefulness requirements** based on the effect of analytical performance on clinical decisions such as:
    a. Toxicological and pharmacological information
    b. Clinicians' opinions related to specific cases
2. Published **professional recommendations** from:
    a. National and international expert bodies and agencies
    b. Expert local groups or individuals
3. **Performance goals set by**:
    a. Regulatory bodies and agencies
    b. Organizers of External Quality Assessment (EQA) schemes
4. Goals based on the current state of the art, which include:
    a. Inter-laboratory comparison programs

---

[3] See CLIA Interpretive Guidelines for Laboratories, Appendix C http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Interpretive_Guidelines_for_Laboratories.html.

[4] The hierarchy is based on the consensus agreement published in the Scandinavian Journal of Clinical and Laboratory Investigation 1999; 59: 585.

[5] Note: When available and appropriate, models higher in the list are preferred to those lower in the list. However, based on requirements needed to maintain accreditation, proficiency testing requirements define the minimum standards for performance. While proficiency testing may be appropriate for some tests, medical usefulness requirements are usually more stringent and may be preferred.

b. EQA or proficiency testing schemes
c. Current published information on methodology

Analytic goal setting for the quantification of drugs for **medical usefulness requirements** typically requires estimates of biological variation (pharmacokinetics) and drug concentration thresholds that guide clinical management. The rarity of human exposure to most of the agents tested by the LRN-C, and the analysis of urine for metabolites compromise the ability to collect the necessary pharmacokinetic/pharmacodynamic modeling (PK/PD) information needed to establish objective medical requirements for method performance.

Published professional recommendations for the accuracy and precision of analytical toxicology methods with intended use comparable to that of the LRN-C are available from the Scientific Working Group for Forensic Toxicology, the Food and Drug Administration, the National Laboratory Certification Program and the Clinical and Laboratory Standards Institute.[vi]

Performance goals set by CDC (in the Public Health Emergency Preparedness cooperative agreement) clearly establish minimum quality requirements for analytical methods. LRN-C Level 1 and 2 laboratories must attain and maintain "qualified" status, (i.e., the laboratory must meet **reference materials characterization requirements and perform successfully in scheduled PT exercises**).

Based on the criteria described above, total allowable error (TE_a), incorporating evaluation of both accuracy and precision, should not exceed 25% for LRN test methods. The TE_a is the maximum error that can be tolerated under terms of the intended use of test findings.

B. Reportable Range

The reportable (or analytical measurement) range is established in each analytical run, through analysis of a full range, multi-point calibration set.[6] The process of confirming that the assay system will accurately determine the concentration of the analyte over the reportable range includes the reprocessing of each calibrator as a sample against the calibration curve. At a minimum, each calibrator must meet the qualitative (e.g., chromatography, ion ratio) and quantitative acceptance criteria used for controls. If a linear calibration model has been established, the linear regression coefficient of determination (r^2) should equal or exceed 0.990.

Test samples exceeding the upper limit of calibration may be diluted to bring the concentration of analyte within the reportable range. An analyte-free matrix should be used to perform dilutions. If an alternate diluent is used, the laboratory should compare results from the analysis of samples diluted with matrix blank and the alternate diluent. Alternative practices to dilution (as done in trace element analysis) include:

- calibration verification using linear dynamic range standards, and
- analysis of characterized materials with similar concentrations to the sample.

Any practice used to "extend" the reportable range must be validated.

[6] Note: The calibration model is established in method development and must not be modified during validation or subsequent use. If the validation data suggest that the calibration model is inappropriate, then a change would be made and the validation process restarted for accuracy/precision.

The LRN does not permit detection or quantification of analyte below the concentration of the lowest calibrator, S1. Trace element methods do not require quantification below S2 if employing the CDC's Inorganic and Radiation Analytical and Toxicological[vii] (IRAT) Branch method calibration scheme. Samples with presumed concentration less than the S1 (S2 in trace element methods) or found not to contain the analyte are reported as "none detected" for the analyte(s). Laboratories may choose to experimentally determine the method LOD and LOQ, and if used for reporting purposes, the laboratory is required to apply appropriate protocols for periodic (twice each year minimum) calibration and reportable range verification.

C. Analytical Sensitivity and Specificity Recommended Practices to Minimize Risks for False Negative Results

- *Define limits of detection and quantitation as the concentration of analyte in lowest non-zero assay calibrator (S1)*. For LC and GC methods, mass spectrometric analysis of S1 should yield a peak with signal-to-noise that equals or exceeds 10; the ion ratio(s) used to identify the analyte must consistently fall within acceptance range(s); and the quality of chromatography (i.e., symmetry and resolution, should provide evidence of a lack of interference). For all methods the back calculation of S1 (S2 may be appropriate for trace elements) analyte concentration using the calibration curve should be in acceptable agreement with the assigned calibrator concentration (e.g., +/- 30%) or a lab-defined fixed number of measurement units (e.g., 1 ug/L).

- *Report findings below the S1 calibrator as "None Detected."* The CDC and LRN have established a calibration range that is presumed to meet the needs of those using LRN services. An identified need to quantify analyte that is lower than the concentration on S1 (S2 in trace element analysis) would require the introduction of an additional calibrator at the lower LOQ, or the use of procedures that allow periodic (twice each year) calibration verification and verification of reportable range. Per LRN policy, results for chromatographic methods that meet Network criteria (e.g., ion ratio, S/N) should be reported as present but lower than the calibrator (i.e. <S1) in Results Messenger.

- *Control and monitor matrix effects.*[7] Specimen extraction procedures should be optimized in method development to minimize the influence of matrix on analyte recovery and the presence of interferents in the analyte detection/quantification. Likewise, extraction and chromatographic conditions should be optimized in method development to minimize signal suppression/enhancement in LC/MS analysis.

  Matrix effects in trace element testing are minimized through the use of appropriate sample diluent components, selection of appropriate IS and isotopes for measurement, use of collision and/or reaction cells, control of oxides and double-charged ions, use of correction equations for isobaric interferences, and optimization of other method characteristics.

---

[7] For the recommended approach to evaluate the potential for components of the sample matrix to either suppress or enhance the ionization of drug and internal standard analytes, see B.K. Matuszewski, M.L. Constanzer, and C.M. Chavez-Eng. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS. Analytical Chemistry. 75: 3019–3030 (2003).

Routine practices to monitor the influence of matrix, including interferents, on analytical performance should include (for each sample) an evaluation of recovery of internal standard from prepared sample, relative to the internal standard abundance in prepared calibrators. Peak symmetry and resolution should be monitored in chromatographic methods. The use of an isotopically-labeled internal standard for individual molecular analytes, and measuring isotopes of appropriate mass and ionization energies for trace elements act to mitigate matrix effects.

- *Store and analyze specimens within limits of specimen and analyte stability*. Emergency response typically requires rapid delivery of newly-collected specimens to the laboratory for immediate analysis. Surge capacity exercises suggest that the time from specimen collection to the completion of analysis is about four days. The laboratory should assess the stability of primary specimen and extract, under conditions of specimen handling and analysis over a minimum interval of four days. Long-term storage stability of primary specimens should be assessed if specimens are archived for repeat analysis. Published evaluations by others performing the same testing elsewhere can be utilized in lieu of an in-house evaluation.

D. Analytical Specificity Recommended Practices to Minimize Risks for False Positive Results

- *For chromatographic methods, use retention time and mass spectrometric response for qualifier ions to definitively identify the presence/absence of analyte(s) of interest.*
    - Recommended practice for GC/MS is the use of relative ion abundance ratios of a minimum three diagnostic (structurally significant) ions (two ion ratios) to identify the analyte. A minimum one qualifier ion for internal standard is recommended. Identification of the analyte in test samples requires ion abundance ratios to fall with 20% of the respective average ratio determined from calibrators.
    - Recommended practice for tandem mass spectrometry is the detection of at least two and preferably three precursor-product ion transition characteristics of each analyte, and relative abundance criteria for the selected ions of 20% of the mean determined from calibrators[8].
    - Recommended retention time criterion for peak identification is CLSI C50-A: relative retention time of the analyte should correspond to that of a contemporary standard at a tolerance of +/- 0.5% for GC and +/- 2.5% for LC; or, CLSI 43-A2: +/- 1% or 0.2 minutes, whichever is smaller; or, NLCP guidance: 2% retention time window around the analyte retention time in a contemporary calibrator.
- *For chromatographic methods, evaluate isotope-labeled internal standard for content of unlabeled analyte*. New lots of internal standard should be analyzed for the presence of non-labeled analyte, and subsequently in each analytical run, blank quality control specimens must be evaluated for the presence of analyte.
- *For all methods, monitor assay performance for carryover*. A recommended practice for the assessment of carryover in each analytical batch is the processing of a blank quality control specimen immediately following the highest calibrator. There should be

[8] Note that alternative criteria for permitted relative ion intensity tolerances based on the relative intensity of the base peak have been published and should be considered where a 20% criterion is proven unacceptable.

no evidence of significant analyte in the blank quality control sample (e.g., ion ratios should not pass acceptance criteria, or the concentration should be determined by the instrument data system as not detected or below laboratory-established thresholds, or signal-to-noise should fail acceptance criteria). Should an injection of a test sample produce a result that exceeds the highest calibrator, the following sample should be re-injected after a blank to assess whether significant carryover occurred.

- *Contamination control*. This is of particular importance for trace element analyses. Possible contamination sources should be minimized by lot-checking of collection and storage containers, transfer equipment, and any other equipment in direct contact with samples. Chemical reagents should also be tested or certified as trace element suitable. Engineering practices (e.g., trace metal-clean preparation environments should be employed).

E. Reference Intervals

The reference interval can be defined as "None Detected" or "Expected concentrations in the population fall below the limit of quantification" for LRN-C core and alternative methods used to **detect compounds not expected to be present in an unexposed population**. The published reference intervals for LRN-C core and alternative methods used to **detect compounds that are normally present in the laboratory's patient population** should be verified for the type of specimen and relevant demographic variables such as age and sex where appropriate.

It is acceptable to use pertinent literature references, such as the National Health and Nutrition Examination Survey or NHANES) or the Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles, to define reference intervals for targets that are normally present in the patient population. Where appropriate, a verification study should include the examination of a small number of specimens (approximately minimum 20) to determine whether more than 10% of the results exceed the published reference interval 95% confidence limits. If the percentage of outliers exceeds 10%, the laboratory must obtain a minimum 120 specimens to establish the reference interval.

LRN-C METHOD PERFORMANCE SPECIFICATIONS AND VALIDATION PLAN

Performance Characteristic	Performance Specification	Validation Practices
Accuracy	12% maximum allowable inaccuracy	Determined by replicate analysis of samples containing known amounts of the analyte. • Accuracy and precision, at a minimum, should be measured using a minimum of five determinations per concentration over five different runs performed over a minimum three days. For established test methods, Laboratory Directors may choose to establish accuracy and precision using historical data (e.g., LRN characterization data) as long as laboratory methods are not modified. • A minimum of three concentrations targeted at 3 x LOQ, mid-range and 80% x ULOL is recommended.
Precision	8% maximum allowable imprecision (may vary among analytes based on state of practice)	

Performance Characteristic	Performance Specification	Validation Practices
Total Allowable Error	25%	Calculated as TEa = Bias (%) + 1.65 x CV (%)
Reportable (Analytical Measurement) Range or AMR	Established by full range multi-point calibration as defined by the LRN-C.	Reprocess each calibrator as a sample against the calibration curve. Each calibrator must meet all chromatographic and identification acceptance criteria, and must recover to within 20% of the calibrator assigned value. Lowest (LOQ) calibrator may recover to within 30% or a fixed number of measurement units, e.g., 1 ug/L, around the assigned concentration. If a linear calibration model has been established, the linear regression coefficient of determination (r2) should equal or exceed 0.990.
AMR	Validate results exceeding AMR	Specimen dilution, calibration verification using linear dynamic range standards as done in trace element analysis or other options must be validated by demonstration of target recovery within acceptable limits described above.
Sensitivity (limits of quantification and detection)	Administratively set to the lowest non-zero calibrator (S1) analyte concentration, or to S2 if working off IRAT trace element methods Alternatively apply statistical criteria to the analysis of blank matrix specimens to derive LOD	Document S1 (S2 trace element) analyte response from each validation run calibration: S/N >10; the back calculated value should be within 30% of assigned value; chromatography and identification criteria satisfied. (Optional experiment: A minimum of three replicates per run of the lowest calibrator or independently prepared specimen may each be analyzed over three runs to demonstrate that all detection and identification criteria are met.)

Performance Characteristic	Performance Specification	Validation Practices
Specificity	Definitive identification of target analyte(s) using chromatographic retention time and mass spectrometric analysis	GC/MS analysis: • determine the relative ion abundance ratios of a minimum three diagnostic (structurally significant) ions (two ion ratios) • relative ion abundance ratios must agree within ±20% of the corresponding ion ratios established by the calibration Tandem Mass Spectrometry analysis: • detect at least two and preferably three precursor-product ion transition characteristics of each analyte • relative abundance criteria for the selected ions must agree within ±20% of the corresponding ion ratios established by the calibration (Note: alternative criteria for permitted relative ion intensity tolerances based on the relative intensity of the base peak may be acceptable.) Retention time criterion for peak identification is a 2% (or laboratory derived criterion based on stable assay performance) retention time window around the analyte retention time in a contemporary calibrator. An alternative is the use of relative retention time window. Specificity for trace elements is optimized by practices such as measuring isotopes of appropriate mass, optimizing instrument conditions such as oxides and double charged ions, and other practices.
Carryover	No evidence of the presence of analyte in blank samples that follow the analysis of samples with analyte concentration at the carryover limit (ULOL)	Process the blank quality control specimen immediately following the highest calibrator in each analytical batch. Verify no evidence of significant analyte in the blank quality control sample: • no significant peak meeting all target identification criteria is present in the analyte retention time window

Performance Characteristic	Performance Specification	Validation Practices
Interference	Matrix Interference: biological matrices used for the detection and quantification of target analytes do not compromise the quality of analyte chromatography or the specificity in analyte detection.	Routine practices to monitor the influence of matrix, including interferents, on analytical performance of chromatographic methods should include for each sample: • an evaluation of peak symmetry and resolution; and, • comparison of each specimen's internal standard (IS) area to the average IS area of calibrators. Specimen IS area outside the range 50% to 200% of the average calibrator IS area is suspect for matrix effect or error in the addition of IS. Optional experiment: Blank matrix samples from a minimum of ten different sources without the addition of an internal standard may be analyzed to demonstrate the absence of common interferences from the matrix.
	Xenobiotic Interference: compounds that are structurally-similar to target analyte do not compromise assay specificity.	Evaluate assay performance when challenged with compounds that are commonly used in the testing population (e.g., over-the-counter drugs, prescription drugs, illicit compounds) that could potentially interfere with the assay.
	Internal Standard: Non-labeled analyte in the internal standard must not cause false positive findings in specimen analysis.	Analyze a blank matrix sample spiked with the internal standard and verify claims made in the certificate of analysis for internal standard purity. Non-labeled analyte introduced by addition of internal standard for specimen analysis should not produce an analyte concentration that exceeds 10% the analyte concentration in the lowest calibrator (S1).
Matrix Effects (Ionization Suppression / Enhancement)	The average suppression or enhancement is less than ±25%, and relative standard deviation of the suppression or enhancement is less than 15%; else, the laboratory demonstrates that there is no impact on analytical sensitivity and accuracy.	The recommended approach to evaluate the potential for components of the sample matrix to either suppress or enhance the ionization of drug and internal standard analytes is in B.K. Matuszewski, M.L. Constanzer, and C.M. Chavez-Eng. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS. Analytical Chemistry. 75: 3019–3030 (2003).

Performance Characteristic	Performance Specification	Validation Practices
Reference Interval (normal values)	The reference interval is defined as "None Detected" for LRN-C core and alternative methods used to detect compounds not expected to be present in an unexposed populations. The published reference intervals for LRN-C core and alternative methods used to detect compounds that are normally present in the laboratory's patient population are verified for the type of specimen and relevant demographic variables such as age and sex. Potential regional differences may also need consideration	If a verification study is required by the laboratory director, a verification study includes the examination of a small number of specimens (minimum 20) to determine whether more than 10% of the results exceed the published reference interval 95% confidence limits. If the percentage of outliers exceeds 10%, the laboratory must obtain a minimum 120 specimens to establish the reference interval. ***Reference range*** means the range of test values expected for a designated population of individuals, e.g., 95 percent of individuals that are presumed to be healthy (or normal). (CLIA'88 definition)
Analyte Stability (Primary and Processed)	Analytes in the primary and processed samples are documented to be stable over the time interval needed to complete analysis.	LRN laboratories typically process specimens within four days of primary specimen receipt. Primary sample stability: • prepare samples fortified with analyte(s) at both low and high concentrations • analyzed in triplicate to establish time zero responses • aliquot the fortified samples into a minimum of four separate storage containers per concentration level • analyze refrigerated aliquots at time = 24 hrs, 48 hrs, 72 hrs and 96 hrs Processed sample stability: • combine extracts of fortified samples into low and high extract pools • divide into a minimum of 10 autosampler vials • immediately analyze first vials of each concentration in triplicate to establish the time zero responses. • Store all remaining vials in a manner that they would typically be stored during routine analysis (e.g., refrigerated, at room temperature on autosampler). • Analyze the remaining vials in triplicate at time intervals 24 hrs, 48 hrs, 72 hrs and 96 hrs Loss of analyte exceeding 10% the concentration at t=0 is evidence of instability.

ACKNOWLEDGEMENTS

The success of this document is due to the following CLIA Validation Taskforce members.

Sanwat Chaudhuri
Utah Public Health Laboratory

Bill Edgemond
Texas Department of State Health Services

Margi Haas
Centers for Medicare and Medicaid Services

Jennifer Jenner
Massachusetts Department of Public Health: William A. Hinton State Laboratory Institute

Dick Jenny
Wadsworth Center, New York State Department of Health

Jeff Moran, Chair
Arkansas Public Health Laboratory

Tonia Parrott
(Formally with the National Center for Environmental Health, CDC)

Noel Stanton
Wisconsin State Laboratory of Hygiene

Bonnie Taffe
Michigan Department of Community Health

Gary Yamamoto
Centers for Medicare and Medicaid Services

APHL RESOURCE

APHL created an LRN-C toolkit[viii] where users have a platform to share templates, forms and other resources to help one another out, as well as a discussion board to post questions to the entire group. The latest feature includes a section where laboratorians can tell APHL when they have information for a story. This and other related documents can be found on the Toolkit.

RESOURCE LINKS

[i] Laboratory Response Network: http://www.bt.cdc.gov/lrn/

[ii] Laboratory Response Network for Chemical Threats: http://www.bt.cdc.gov/lrn/chemical.asp

[iii] Clinical Laboratory Improvement Amendments: http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/index.html?redirect=/clia/

[iv] Association of Public Health Laboratories: http://www.aphl.org/Pages/default.aspx

[v] National Standards for Public Health Preparedness at the State and Local Level: http://www.cdc.gov/phpr/capabilities/dslr_capabilities_july.pdf

[vi] Public Professional Recommendations:
Scientific Working Group for Forensic Toxicology: http://www.swgtox.org/
Food and Drug Administration: http://www.fda.gov/
National Laboratory Certification Program: http://www.workplace.samhsa.gov/DrugTesting/Level_1_Pages/NLCP%20Program%20Documents.html
Clinical and Laboratory Standards Institute: http://www.clsi.org/

[vii] CDC's Inorganic and Radiation Analytical and Toxicological: http://www.cdc.gov/nceh/dls/iratb.html

[viii] LRN-C toolkit: https://www.aphlweb.org/aphl_departments/Environmental_Health/Environmental_Health_Program/chemt/LRNCToolkit/default.aspx (Requires permission to access this resrouce. For more information, contact EH@aphl.org)

Association of Public Health Laboratories

The Association of Public Health Laboratories (APHL) is a national nonprofit dedicated to working with members to strengthen laboratories with a public health mandate. By promoting effective programs and public policy, APHL strives to provide public health laboratories with the resources and infrastructure needed to protect the health of US residents and to prevent and control disease globally.

This publication was 100% financed by federal funds. The total amount of funding received for the EH Program is $679,747. This publication was supported by Cooperative Agreement # U60HM000803 from CDC. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC.

8515 Georgia Avenue, Suite 700
Silver Spring, MD 20910
Phone: 240.485.2745
Fax: 240.485.2700
Web: www.aphl.org