THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

ETV Verification Statement

TECHNOLOGY TYPE:	**QUALITATIVE SPOT TEST KIT**
APPLICATION:	**LEAD-BASED PAINT DETECTION**
TECHNOLOGY NAME:	**Lead-in-Paint Test Kit**
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The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies. Information and ETV documents are available at www.epa.gov/etv.

ETV works in partnership with recognized standards and testing organizations, with stakeholder groups (consisting of buyers, vendor organizations, and permitters), and with individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field and laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted according to rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

This verification test was conducted under the U.S. EPA through the ETV program. Testing was performed by Battelle, which served as the verification organization. This verification test was conducted in response to the call of the Renovation, Repair, and Painting (RRP) rule for an EPA evaluation and recognition program for test kits that are candidates to meet the false positive and negative goals of this rule. Per the RRP rule, a test kit should have a demonstrated probability (with 95% confidence) of a false negative response less than or equal to 5% of the time for paint containing lead at or above the regulated level, 1.0 mg/cm^2 and a demonstrated probability (with 95% confidence) of a false positive response less than or equal to 10% of the time for paint containing lead below the regulated level, 1.0 mg/cm^2. Battelle evaluated the performance of qualitative spot test kits for lead in paint. This verification statement provides a summary of the test results for ANDalyze, Inc. Lead-in-Paint Test Kit.

TECHNOLOGY DESCRIPTION

Following is a description of the ANDalyze Lead-in-Paint technology, based on information provided by the vendor. The information provided below was not verified in this test.

The ANDalyze Lead-in-Paint Test Kit utilizes a sensor/fluorimeter platform to quantitatively detect lead in paint. The test is based on a sensing technology which uses special DNA sequences called DNAzymes (DNA enzymes) that specifically bind to lead ion (Pb^{2+}) and are capable of performing catalysis. The DNA sequence is linked to fluorophores/quencher pair. The fluorescence of the fluorophore is quenched due to its close proximity to the quencher. In the presence of lead, the DNAzyme catalyzes the cleavage of the substrate strand which releases the cleaved fragment containing the fluorophore into solution thereby enhancing the fluorescence. The increased level of fluorescence upon reaction with lead can be measured using a fluorimeter. The rate of this increase is proportional to the lead concentration.

To extract soluble lead (as Pb^{2+}) from a dry paint surface, a 1.2 cm^2 area of paint is either drilled using a Craftsman drill fitted with a ½ inch drill bit or cut using a razor blade. The entire paint sample is transferred into a plastic tissue grinder to which 2 mL 25% nitric acid is added. The paint chips are then ground to a fine powder by rotating the pestle of the tissue grinder for approximately 2 – 5 minutes which results in Pb^{2+} being extracted into the acidic solution. The test is performed by first transferring and mixing 10 µL volume of the acidified Pb^{2+} extract into the testing buffer. This is the test solution. A glass tube is inserted into the sample chamber of the fluorimeter and sensor housing is placed on the glass tube. Using a syringe, 0.7 mL of test solution is withdrawn and pushed through the sensor housing into the glass tube. The lead reacts with the DNA-based sensor during this step. The housing is immediately removed, the lid is closed and the START button is pressed. The lead concentration in paint is displayed on the screen within 30 seconds in units of mg/cm^2.

At the time of testing, a test kit included a fluorimeter at $1500 and 50 test consumables at $300. Refill consumables could be purchased for further testing. Optional: A Craftsman drill could be purchased from ANDalyze at a cost of $310 if the user does not own one. The ANDalyze fluorimeter could be used for any other tests which utilize fluorescent sensing methods.

VERIFICATION TEST DESCRIPTION

This verification test of the ANDalyze Lead-in-Paint Test Kit was conducted January through June 2010 at the Battelle laboratories in Columbus, Ohio. This timeframe included testing of the test kit and completion of all ICP-AES and QC analyses.

Qualitative spot test kits for lead in paint were evaluated against a range of lead concentrations in paint on various substrates using performance evaluation materials (PEMs). PEMs were 3-inch by 3-inch square panels of wood (pine and poplar), metal, drywall, or plaster that were prepared by Battelle. Each PEM was coated with either white lead (lead carbonate) or yellow lead (lead chromate) paint. The paint contained lead targeted at 0.3, 0.6, 1.0, 1.4, 2.0, and 6.0 mg/cm^2. These lead concentrations were chosen with input from a stakeholder technical panel based on criteria provided in EPA's lead Renovation, Repair, and Painting (RRP) rule and to represent potential lead levels in homes. Paint containing no lead (0.0 mg/cm^2) was also applied to each substrate and tested.

Two different layers of paint were applied over the leaded paint. One was a primer designed for adhesion to linseed oil-based paint and the second coat was a typical interior modern latex paint tinted to one of three colors: white, red-orange, or grey-black. These colors were chosen by EPA, with input from a technical stakeholder panel, based on the potential of certain colors to interfere with lead paint test kit operations. The top-coat paint manufacturers' recommended application thickness was used. Two coats at the recommended thickness were applied.

The ANDalyze Lead-in-PaintTest Kit for lead paint was operated by a technical and non-technical operator. The technical operator was a Battelle staff member with laboratory experience who had been trained by the vendor to operate the test kit. The same technical operator operated this test kit throughout testing. Because this lead paint test kit is anticipated to be used by certified remodelers, renovators, and painters, it was also evaluated by a non-technical operator. The non-technical operator was a certified renovator with little-to-no experience with lead analysis. The non-technical operator was provided the instruction manual, demonstrational DVD, and other materials (operational tip sheet, material safety data sheets [MSDS]) typically provided by the vendor with the test kit for training. He then viewed the materials himself to understand how to operate the test kit. He was also permitted to ask questions or clarifications of the vendor on the operation of the test kit. This scenario approximated the training that renovators are expected to receive under the RRP rule.

Tests were performed in duplicate on each PEM by each operator, technical and non-technical. Duplicates were tested in succession by each operator on a given PEM. PEMs were analyzed blindly. Test kit operators were not made aware of the paint type, lead level, or substrate of the PEM being tested. PEMs used for analysis were marked with a non-identifying number. PEMs were not tested in any particular order. To determine whether the substrate material affected the performance of the test kits, two unpainted PEMs of each substrate were tested using each test kit, in the same manner as all other PEMs (i.e., per the test kit instructions). Three PEMs at each lead level, substrate, and topcoat color were prepared for use in this test. Thus, a total of 468 painted PEMs were used in the verification test.

To confirm the lead level of each PEM used for testing, paint chip samples from each PEM were analyzed by a National Lead Laboratory Accreditation Program (NLLAP) recognized laboratory, Schneider Laboratories, Inc., using inductively coupled plasma-atomic emission spectrometry (ICP-AES) as the reference method. The paint chip samples for reference analyses were collected by Battelle according to a Battelle standard operating procedure (SOP), which was based on ASTM E1729. Lead levels determined through the reference analysis were used for reporting and statistical analyses.

The Lead-in-Paint Test Kit was verified by evaluating the following parameters:

- ***False positive and negative rates*** – A false positive response was defined as a positive result when paint with a lead concentration $\leq$0.8 mg/cm^2 was present. A false negative response was defined as a negative response when paint with a lead concentration $\geq$1.2 mg/cm^2 was present. Consistent with the EPA's April 22, 2008 RRP rule, panels with lead levels between 0.8 and 1.0 mg/cm^2 were not used in the false positive analysis, and those with lead levels between 1.0 and 1.2 mg/cm^2 were not used in the false negative analysis..
- ***Precision***– Measured by the reproducibility of responses for replicate samples within a group of PEMs. Groups of PEMs evaluated for precision included lead concentrations and substrate material. Responses were considered inconsistent if 25% or more of the replicates differed from the response of the other samples in the same group of PEMs
- ***Sensitivity*** – The lowest detectable lead level by the test kit. This parameter was identified based on the detection results across all PEM levels and was determined based on the lowest PEM lead level with consistent (>75%) positive responses.
- ***Modeled Probability of Test Kit Response*** – Logistic regression models were used to determine the probabilities of positive or negative responses of the test kit at the 95% confidence level, as a function of lead concentration and other covariates, such as substrate type, lead paint type, operator type, and topcoat color. To account for the uncertainty associated with measurement error of the PEMs, the final multivariable model for each test kit was subjected to a simulation and extrapolation (SIMEX) analysis.
- ***Matrix Effects*** – Covariate adjusted logistic regression models were used to determine whether any of the PEMs parameters (topcoat color, substrate, operator, or lead paint type) affected the performance of the test kit. Type III Statistics and comparison of likelihoods from logistic regression models were used to determine the statistical significance of these factors.

- ***Operational Factors*** – Ease of use, operator bias, helpfulness of manuals, technology cost, and sustainability metrics such as volume and type of waste generated from the use of the test kit, toxicity of the chemicals used, and energy consumption were noted and summarized.

QA oversight of verification testing was provided by Battelle and EPA. Battelle and EPA QA staff conducted technical systems audits and a data quality audit of at least 10% of the test data to ensure that data quality requirements were met. This verification statement, the full report on which it is based, and the test/QA plan for this verification test are available at www.epa.gov/etv/este.html.

VERIFICATION RESULTS

False Positive/Negative Rates: Observed false negative rates for the ANDalyze Lead-in-Paint Test Kit on PEMs with confirmed lead levels of $\geq$ 1.2 mg/cm^2 were 9% overall for the technical operator. Observed false negative rates for the non-technical operator were 12% overall. The observed false negative rates for different substrates and topcoat colors were similar to the overall rates found for each operator.

The overall observed false positive rate for the ANDalyze Lead-in-Paint Test Kit on PEMs with confirmed lead levels of $\leq$ 0.8 mg/cm^2 was 4-5% for both the technical and non-technical operator. The observed false positive rates across different PEM factors (e.g., substrate type, topcoat color, lead paint type) were similar to the overall rates and were similar between the two operators. Observed false positive rates were 10% or lower in all cases.

***Precision*:** The ANDalyze Lead-in-Paint Test Kit produced consistent responses (either positive or negative) across all substrates and paint types at all lead levels except one; the ANDalyze Lead-in-Paint Test Kit results were inconsistent at only 1.0 mg/cm^2.

Results from the ANDalyze Lead-in-Paint Test Kit indicated 100% precision on PEMs that contained no lead. The precision observed when the kit was operated by the non-technical operator was higher than that of the technical operator on white lead PEMs (85% vs. 73%), while the results were reversed for the yellow lead PEMs, with the technical operator having a precision of 81% and the non-technical operator having a precision of 66%. The overall precision across both operators was similar (79% and 73%) for both lead paint types.

***Sensitivity*:** Across all lead paint types and operators, the lowest lead level for which the ANDalyze Lead-in-Paint Test Kit generated consistently positive results was 1.4 mg/cm^2 lead. When sensitivity was evaluated by operator type, consistently positive results were found at 1.4 mg/cm^2 on white and yellow overall for the technical operator. Consistently positive responses were found at the 2.0 mg/cm^2 lead level for the technical operator on white lead PEMs and the 1.4 mg/cm^2 lead level for yellow lead PEMs. The overall sensitivity as determined through evaluations performed by the non-technical operator was at the 2.0 mg/cm^2 lead level. This is higher than the sensitivity determined by the technical operator.

***Modeled Probability of Test Kit Response*:** Based on the lower bound estimates of the modeled probability of the ANDalyze Lead-in-Paint Test Kit, the technology did not meet the false negative criterion ($\leq$5%) at the 1.2 mg/cm^2 lead level. False negative rates were predicted to range from 45.0 to 74.5%. Based on the upper bound estimates of the modeled probability of the ANDalyze Lead-in-Paint Test Kit, the technology did not meet the false positive criterion ($\leq$10%) at the 0.8 mg/cm^2 lead level. The lowest false positive rate expected to be achieved at this lead level is 19.6% for a metal substrate with a red topcoat; the highest false positive rate expected to be achieved at this lead level is 45.2% for a plaster substrate with white topcoat.

***Matrix Effects*:** After controlling for the significant covariates, the likelihood of a positive test result is positively and significantly associated with higher lead levels, drywall, metal and plaster substrates, and grey and white topcoats. It is not significantly and positively associated with red topcoats or wood substrates.

Operational Factors: The technical operator found the ANDalyze Lead-in-Paint Test Kit instructions to be clear, informative, and easy to follow. The non-technical operator, however, did not. Both the technical and non-technical operator stated that a significant amount of training and possibly previous experience or laboratory knowledge would be needed to successfully operate this test kit.

All reagents came prepared and ready to use. The solutions used for different steps were easily identifiable within the kit. Storage conditions of the reagents were not marked on the containers, although the ANDalyze Lead-in-Paint Test Kit instruction manual did indicate storage requirements and a temperature range for test kit operation.

The ANDalyze Lead-in-Paint Test Kit, as supplied for this verification test, included a drill, modified half-inch drill bit, fluorimeter, razor blades, ruler, 15 mL grinding tubes and pestles, nitric acid, plastic pipettes, calibration solution and buffers, sensor housings, 30 mL plastic tubes pre-filled with testing buffer, 10 x 76 millimeter (mm) glass test tubes, 1 mL syringes, 10 μL mini-pipette and disposable tips, and grinder cleaning solution.

The waste generated for this test kit included both liquid and solid waste. Solid waste included pipette tips, glass test tubes, housing sensors, plastic tubes, and disposable plastic pipettes. Liquid waste included approximately 2 mL of ground paint in nitric acid; 20 mL of testing buffer, 3 mL of calibration solutions, and 2-3 mL of cleaning solution.

Operation of the test kit took approximately 18 minutes by both the technical and non-technical operator, not including the grinder washing procedure. A normal power supply was needed for the operation of the fluorimeter.

Signed by: Sally Gutierrez – December 03, 2010

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Sally Gutierrez Date
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