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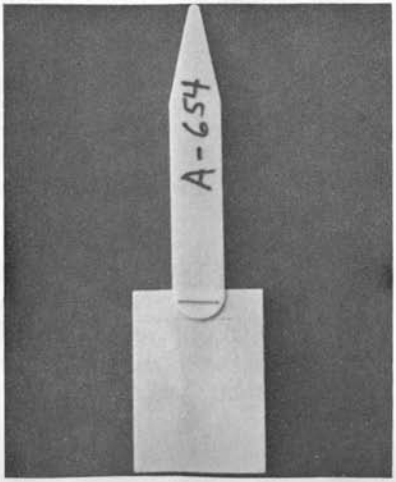

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Cover Photo: Parent hands test sample to volunteer of the American Friends Service Committee during demonstration of dipstick test for lead poisoning. Insert shows photo of actual dipstick.

FOREWORD

Why research on lead?

Perhaps, it seems strange that The Connecticut Agricultural Experiment Station would do research on how to find lead in children's bodies. The answer is simple, I think. We can put our chemical expertise to work on an important problem in contemporary society.

We began as chemists nearly a century ago. What could chemistry do for society? We continually ask that question. We began by showing that a chemist could tell Connecticut farmers the difference between Quinnipiac River mud and Peruvian guano as fertilizers. We went on to use chemistry to tell the difference between pure food and adulterated food. We administered perhaps the first pure food law in the country.

Later our chemists went pioneering the field of human nutrition. We proudly display gold medals for discovering the significance of amino acids in the diet and the first vitamin.

Children eat lead paint. This is nutrition, abrnormal nutrition, but nutrition withal. Thus, it came within our purview. How can a chemist tell if a child has eaten lead? If the child has eaten lead, he secretes an uncommon amino acid into his urine. The chemist can find it. Lead blocks the transformation of the amino acid in the child's body. Thus, the amino acid piles up, and the child excretes it with his urine — the more the amino acid, the more lead he has eaten.

We discussed the matter with the Department of Health in Hartford and with their help developed a rapid assay method. The paper presented here tells the story. Now the fight against lead has a new weapon. We still don't know why a child eats lead, but at least we can now more easily tell if he has.

JAMES G. HORSFALL
Director

CONTENTS

The authors: Dr. Lester Hankin and Dr. Kenneth R. Hanson are Biochemists in the Department of Biochemistry at The Connecticut Agricultural Experiment Station. Dr. Joseph M. Kornfeld is Research Microbiologist and Dr. William W. Ullmann, Director, the Laboratory Division of the Connecticut State Department of Health.

SUMMARY

A new method for the mass screening of children for lead poisoning is described in this Bulletin. The method, based on the determination of delta-aminolevulinic acid in urine, employs a dipstick consisting of a piece of cation exchange paper stapled to a plastic handle. The paper is dipped into the urine sample, air dried, and mailed to the laboratory. As the non-laboratory operations can be performed by a parent or social worker, the problem of collecting and delivering large numbers of urine samples to the laboratory is simplified. Procedures in the laboratory are less cumbersome than those presently used and allow many more samples to be examined. The test is designed for mass screening and many children not now being tested can be examined. Satisfactory correlations were found between assay values determined by the dipstick procedure and by the column method presently used by the Connecticut State Department of Health. Results of the dipstick test are graded as normal, trace, or positive on the same basis as the column test.

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INTRODUCTION

Lead poisoning is a very serious environmental and public health problem, principally among young children. The most common source of lead is from paint chips eaten by the child. Excessive eating of nonedible materials is called pica. Unless this lead poisoning is diagnosed early, neurological disorders, mental retardation, and even death can result. "High risk" areas for lead poisoning are generally in the inner city where older housing prevails and children have easy access to flaking paint. Victims are usually between the ages of 1 and 6. From 3 to 6 months of fairly steady ingestion of lead is necessary before clinical symptoms develop. Therefore it is important to test children for chemical evidence of potential poisoning so that remedial therapy can be undertaken before the child is permanently impaired. A number of laboratory tests have been useful in determining lead intoxication prior to the appearance of clinical symptoms.

Because lead intoxication precedes clinical symptoms it is important to screen large numbers of children on a continuing basis. Children in "high risk" areas who indulge in pica are in constant danger, and an initial negative test for lead poisoning does not guarantee that they may not be poisoned later. Two methods of laboratory testing are generally used: lead in blood (1) and delta-aminolevulinic acid (ALA) in urine (2). The relative clinical and diagnostic merits and disadvantages of each have been discussed (3, 4, 5). The determination of lead in blood samples requires at least a 5 ml sample, and the blood must be drawn by a physician or highly trained technician. A second person is frequently needed to restrain or reassure the child. Such a method is clearly not ideal in a mass screening program. The equipment needed for the direct determination of lead in blood is also expensive. The second method, the assay of ALA in urine, is based upon the fact that ingested lead interferes with hemoglobin synthesis in which an enzyme called delta-aminolevulinate dehydratase is essential. Lead reduces the activity of this enzyme, ALA accumulates, and then abnormal amounts are excreted in the urine. Excessive ALA in the urine is therefore an indication of lead ingestion. Samples of urine can be collected by nonprofessionals.

As part of a program for the detection of lead intoxication, the State of Connecticut tests urine for ALA. In this testing program a major gap exists between the number of children who *should be* tested and the numbers that *are* examined with the resources available. One estimate is that

only about 10% of Connecticut children in the "high risk" age group have been tested even once. The gathering of urine samples by public health officials or their designates, and the prompt transport of these samples to the laboratory consumes a great deal of manpower. The problem would obviously become even more complex if an attempt were made to test the same children regularly. And, the laboratory procedure, using an ion exchange resin column, is somewhat cumbersome and relatively time consuming.

Thus it is clear that present procedures are far from ideal for mass screening programs. The test for ALA in urine as a measure of lead ingestion should meet the following criteria:

1. Sample may be taken by an untrained person.
2. Sampling should cause no pain or trauma to the patient.
3. The sample must not be subject to deterioration, and be easily mailed to the testing laboratory.
4. The method must not generate false negatives.
5. The laboratory and collection procedure must be so simple that frequent retesting (even monthly) is feasible.

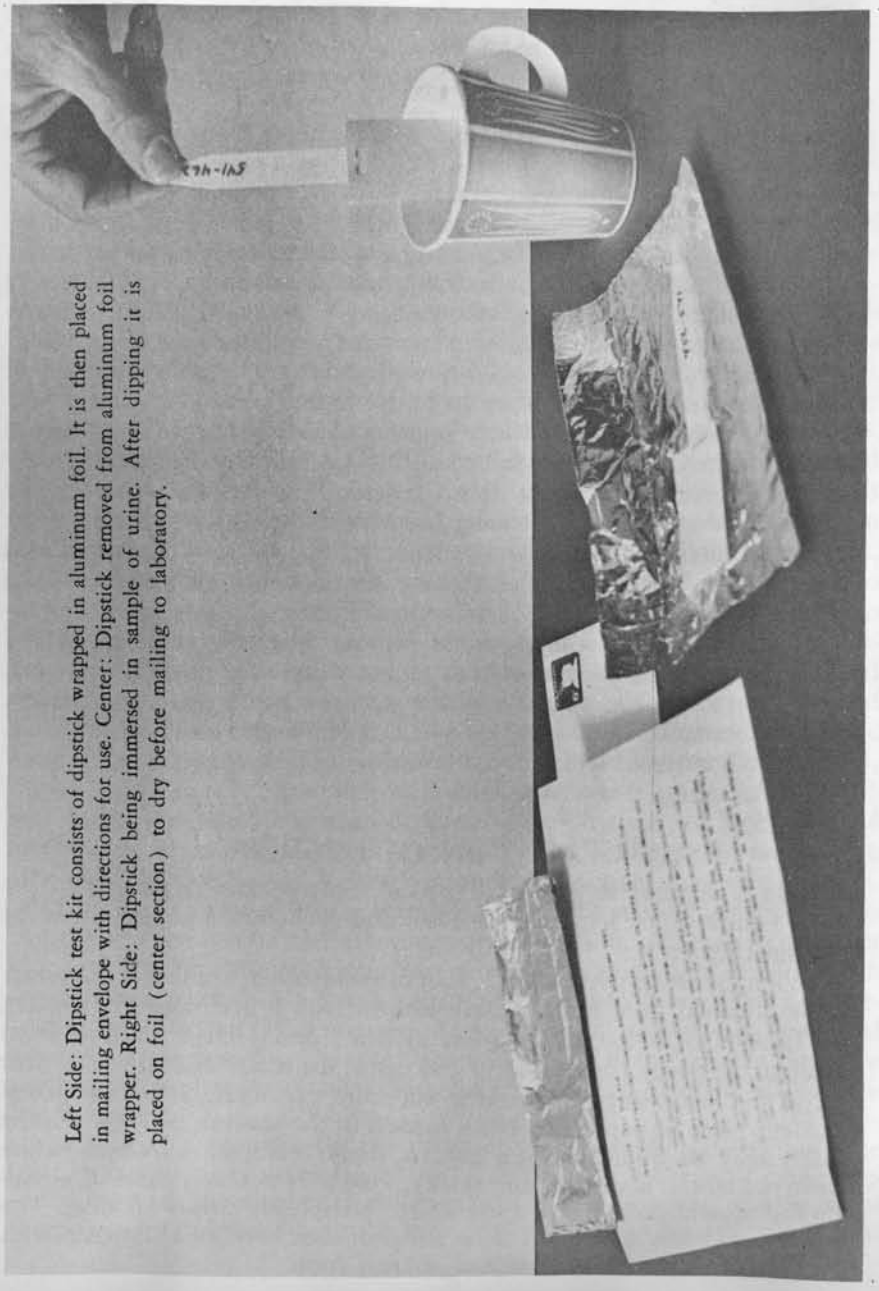
The dipstick test described here appears to satisfy all of these criteria. We first describe the preparation of dipsticks and give directions for their use. The laboratory procedure also is described for those who may be involved in that phase of a testing program. Next we include data from a testing program conducted in a Connecticut inner city. Finally we discuss the need for persons in "high risk" areas to become involved in a mass screening program for lead poisoning in children.

Our major concern in this research was to change the existing method of sampling and sample transmittal so that more children will be tested. In addition, a simplification of the laboratory procedure also was achieved. For a discussion of the laboratory portion of this study and its rationale, the reader is referred to Clinical Pediatrics (6). However, a field test of the dipstick and the results obtained are discussed.

PREPARATION OF DIPSTICKS

Dipsticks are easy to prepare. They are constructed of a piece of paper loaded with a cation ion-exchange resin and stapled to a plastic holder as a handle (Figure 1).

The ion exchange paper (SA-2, sulfonic acid cation exchange, Na form) can be obtained from H. Reeve Angel Co., Inc., 9 Bridewell Pl., Clifton, New Jersey, 07014, as 6 x 4 cm pieces. Draw a pencil line 1 cm down from the top of the paper (see Figure 1) and staple the plastic holder at the 1-cm line. In the laboratory, the paper is cut from the holder along the pencil line. This piece of paper, 5 x 4 cm, is used in the analysis of ALA. Plastic holders may be obtained from garden supply companies (4-inch white plastic pot labels, about .05 mm thick). Fold a 7- x 5-inch piece of aluminum foil around each dipstick so that foil completely covers all sides. The dipstick may then be placed in a self-addressed envelope together with directions for use and a name and address form.



Left Side: Dipstick test kit consists of dipstick wrapped in aluminum foil. It is then placed in mailing envelope with directions for use. *Center:* Dipstick removed from aluminum foil wrapper. *Right Side:* Dipstick being immersed in sample of urine. After dipping it is placed on foil (center section) to dry before mailing to laboratory.

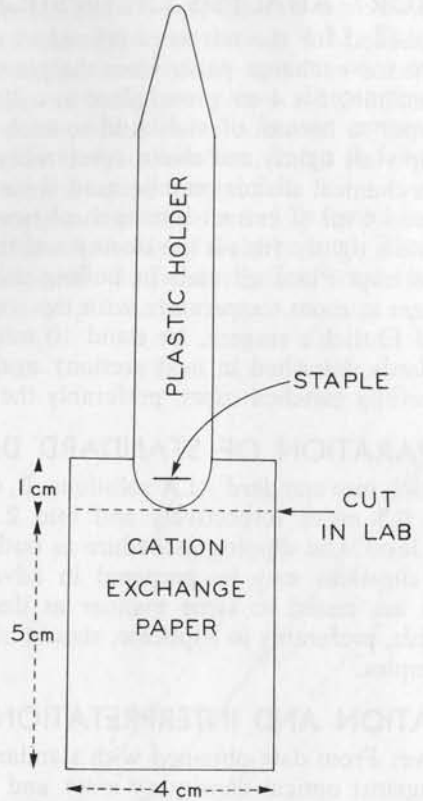


Figure 1. Drawing of dipstick showing dimensions of paper and holder.

FIELD INSTRUCTIONS FOR USE OF DIPSTICK

READ ALL INSTRUCTIONS THROUGH BEFORE USING DIPSTICK.

1. Fill out sheet with name and address of child and name of family physician or clinic.
2. Have child urinate into clean dry container such as a bottle or paper or plastic cup. Use first urine in the morning if possible.
3. Carefully unfold aluminum foil from around dipstick and smooth aluminum foil.
4. Grasp white plastic handle and dip the paper part of dipstick into the urine, making sure that all of the paper gets wet.
5. *Quickly* remove dipstick from urine and brush paper along inside lip of bottle or cup to remove excess urine.
6. Place wet dipstick on the aluminum foil and let it dry. Two hours is usually enough. Paper need not be completely dry.
7. When the paper is dry, fold aluminum foil back around the dipstick, place in envelope together with name-and-address form and mail.

LABORATORY ANALYSIS OF DIPSTICKS FOR ALA

The reagents needed for the test are outlined in the Appendix. In the laboratory, cut the ion exchange paper from the plastic holder at the 1-cm line. Pleat the remaining 5 x 4 cm piece, place in a 20- x 125-mm screwcap vial and push paper to bottom of vial. Add to each vial 10 ml of 0.5 M acetate buffer, cap vials tightly and shake (preferably slowly in a 90° arc) for 20 min. A mechanical shaker may be used if its action is slow. After the shaking, pipette 4 ml of extract into each of two clean vials. Cap one of the duplicate vials tightly (this is the blank) and to the other add 0.1 ml acetylacetone and cap. Place *all* vials in boiling water bath for 20 min, and then cool them to room temperature with tap water. To each vial add 4 ml of modified Ehrlich's reagent, let stand 10 min and read each tube (including standards described in next section) against its own blank at 553 nm. Use carefully matched tubes, preferably the same two tubes.

PREPARATION OF STANDARD DIPSTICKS

Immerse dipstick into standard ALA solutions, B, C, D to provide levels of 1.5, 1.0 and 0.5 mg% respectively and into 2.5% urea solution to provide 0 mg% level. Use dipping procedure as outlined for field instructions. Standard dipsticks may be prepared in advance and stored dry. These standards are tested in same manner as described for unknown samples. Standards, preferably in triplicate, should be run with each series of unknown samples.

CALCULATION AND INTERPRETATION OF RESULTS

Standard Curve: From data obtained with standard dipstick, plot mg% ALA (x axis) against optical density (y axis) and draw line of best fit.

Unknowns: Read mg% directly from standard curve. 0 to 0.5 mg% is considered normal. 0.5 to 0.85 mg% is considered to be in the trace range and child should be rechecked. Any value above 0.85 mg% is considered to be positive and a physician should be notified so that further laboratory and clinical tests may be made.

FIELD TEST OF DIPSTICK METHOD FOR ALA IN URINE

Urine samples submitted by public health officials and those collected by volunteers in an inner city were examined by both the column method (2, 7) and by the new dipstick test for ALA. Ninety samples were thus analyzed and a statistical analysis made (8). With the column method a value of 0 to 0.5 mg% of ALA in urine is considered normal, 0.5 to 1.0 mg% as trace and anything above 1.0 as positive. For the dipstick test we have provisionally adopted limits of 0 to 0.5 mg% as normal, 0.5 to 0.85 as trace and above 0.85 as positive. We believe that lowering the positive cut-off point to 0.85 mg% allows for a greater margin of safety. These limits are subject to modification in the light of further testing and widespread use.

The comparison of the 90 samples examined by the column and dipstick tests is shown in Figure 2. The dotted lines in the figures indicate the

standard deviation. Arrows show where the data were divided for statistical analysis. The slope of the regression line is 1.22. This shows that the dipstick test may overestimate the ALA content of some samples when compared to the column method. In any case, a screening program should always have some controls. Samples with known concentrations of ALA should move through the entire procedure, home to laboratory, to insure that standards once achieved are maintained.

Perhaps the most important point in a test designed for mass screening is that no false negative values should be generated. That is, no poisoned person shall go undiscovered. No false negatives were found in this study

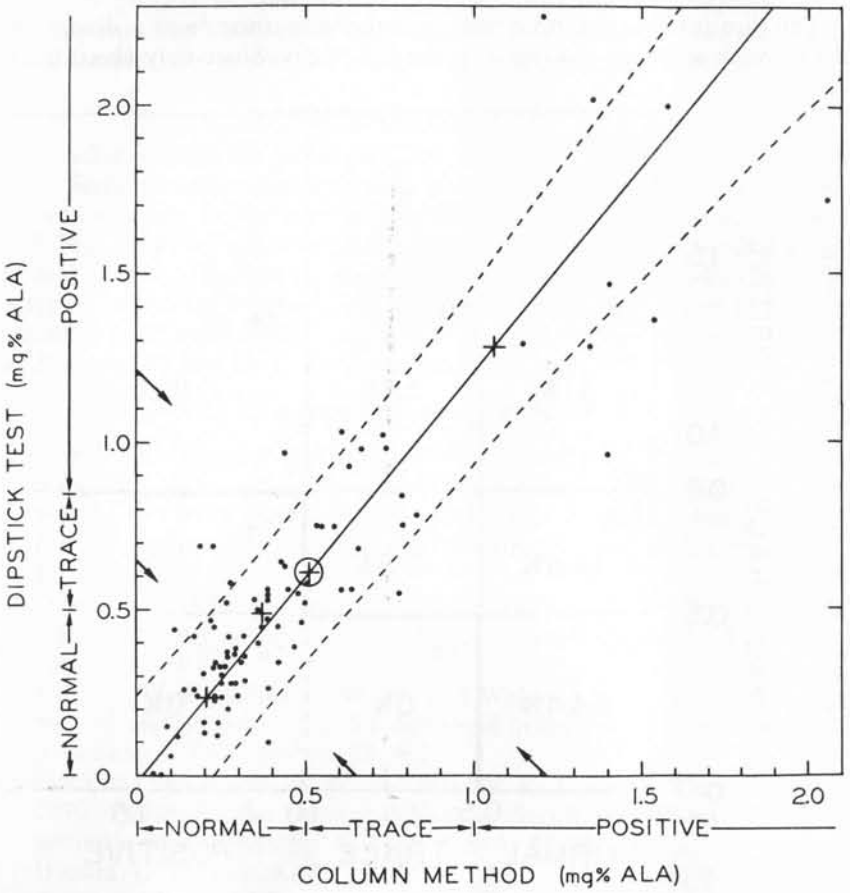


Figure 2. Comparison of the dipstick and column methods for determining delta-aminolevulinic acid in urine. Number of samples = 90 (one point not shown in figures but included in calculations). The solid line indicates a line, fitted by the Bartlett procedure (8), relating the two sets of results. The dotted lines indicate the standard error in Y at various values of X. Open crosses represent the means of the three groups used in the calculations and the circled cross is the mean of the combined group. Arrows indicate where the observations were divided for statistical analysis.

as determined by the procedure shown in Figure 3. The data on samples examined shown in Figure 2 were divided into 9 possible combinations of results shown in Figure 3. The percentage of samples giving the same, or different, results by both tests is recorded. The test limits are the same as in Figure 2. For example, the box labeled $^{-}/_{-}$ indicates those samples which were negative by both tests, and that box designated as $^{tr}/_{-}$ indicates samples giving "trace" by the dipstick test and a negative value by the column method. If there had been any false negative values they would have appeared in the three lower righthand boxes labeled $^{-}/_{tr}$, $^{tr}/_{+}$, and $^{-}/_{+}$. That no sample fell into any of these categories clearly indicates no false negatives were generated by the dipstick test.

The dipstick has also been used by squeezing urine from a diaper onto the dipstick and then placing it on the foil to dry. Since only about 0.6 ml

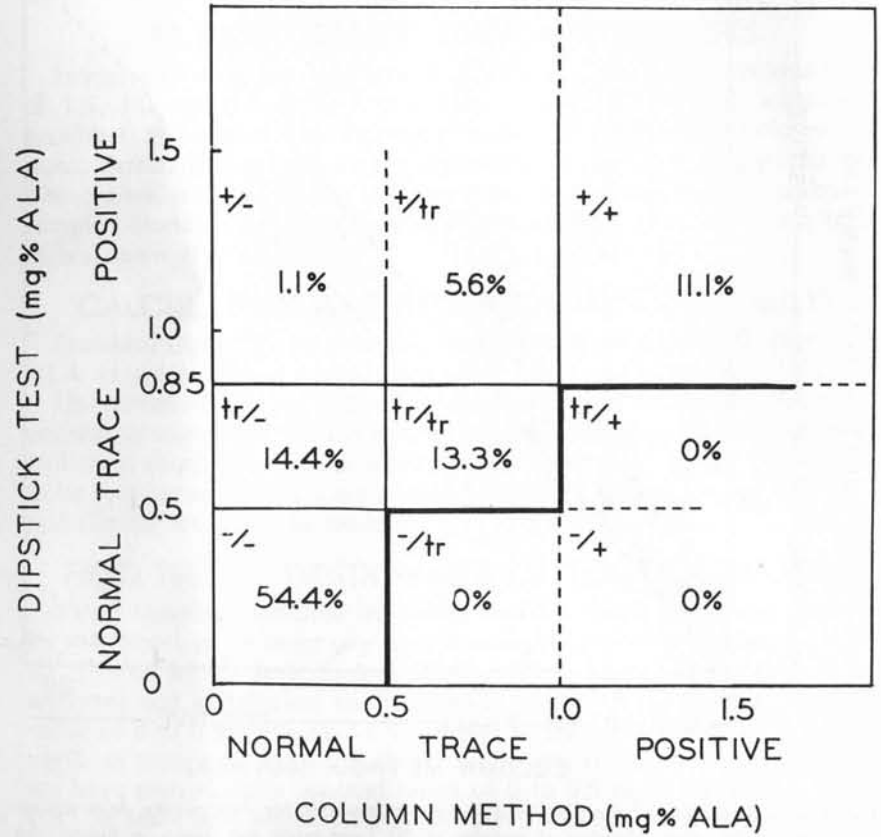


Figure 3. Box score for the data shown in Figure 2 expressed as a percentage of the total number of samples. The 90 samples were graded as positive (+), trace (tr), or normal (—) by each test according to the indicated ALA cut-off point for probable lead ingestion and then assigned to the appropriate box, e.g., dipstick test positive and column method trace is recorded as $+/_{tr}$).

of urine is needed to wet the paper, this technique can be most useful when a more substantial specimen cannot be obtained. We foresee no drawback to this method of collection although the data necessary for validation of this technique are unavailable at this time.

IMPLICATIONS OF TESTING

Up to now, only about a tenth of the children who should be tested for lead poisoning have been examined, including those in the "high risk" areas. The difficulty of analysis and collection of samples has hindered testing. The dipstick test should remove some of this hindrance. First, the laboratory process is speeded and more samples can be accepted. Since many parents can themselves take samples and certainly volunteers can collect samples, the number of collectors can be increased indefinitely.

ACKNOWLEDGEMENTS

We acknowledge the technical staffs of the Biochemistry Department of this Station and the Research Section of the Laboratory Division of the Connecticut State Department of Health for their immense help. We also thank Mahlon Hale and volunteers of the American Friends Service Committee who tested the dipsticks in the field and Dr. C. I. Bliss who suggested the type of statistical analysis used. We thank the J. B. Lippincott Company, publishers of Clinical Pediatrics, for permission to reproduce the figures in this Bulletin. Photos by I. Robinson and I. Zelitch.

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APPENDIX

Reagents needed for the laboratory testing of dipsticks for delta-aminolevulinic acid.

0.5 M Acetate Buffer, pH 4.6 — To about 700 ml. distilled water add 28.5 ml. glacial acetic acid and 68.8 g. sodium acetate trihydrate. Adjust to pH 4.6, dilute to 1 liter with distilled water and store under refrigeration.

Ehrlich's Reagent, modified — To about 100 ml. glacial acetic acid add 5.0 *p*-dimethylaminobenzaldehyde (AR) and 40 ml. of 70% perchloric acid. Dilute to 250 ml. with glacial acetic acid. Prepare fresh daily.

2.5% Urea solution — Dissolve 25 g. urea (AR grade) in distilled water, dilute to 1 liter and store under refrigeration.

Acetylacetone (2,4-pentanedione) — practical grade or equivalent.

delta-Aminolevulinic acid hydrochloride — crystalline, anhydrous.

Stock ALA solutions:

A. Dissolve 64 mg. ALA-HC1 in 2.5% urea solution and dilute to 50 ml. with urea solution.
B. Dil 1.5 ml. A to 100 ml. with urea solution (1.5 mg% level).
C. Dil 1.0 ml. A to 100 ml. with urea solution (1.0 mg% level).
D. Dil 25 ml. C to 50 ml. with urea solution (0.5 mg% level).

Volunteer of the American Friends Service Committee talks to parent, one of many who agreed to have their children tested for lead poisoning by the dipstick test.

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