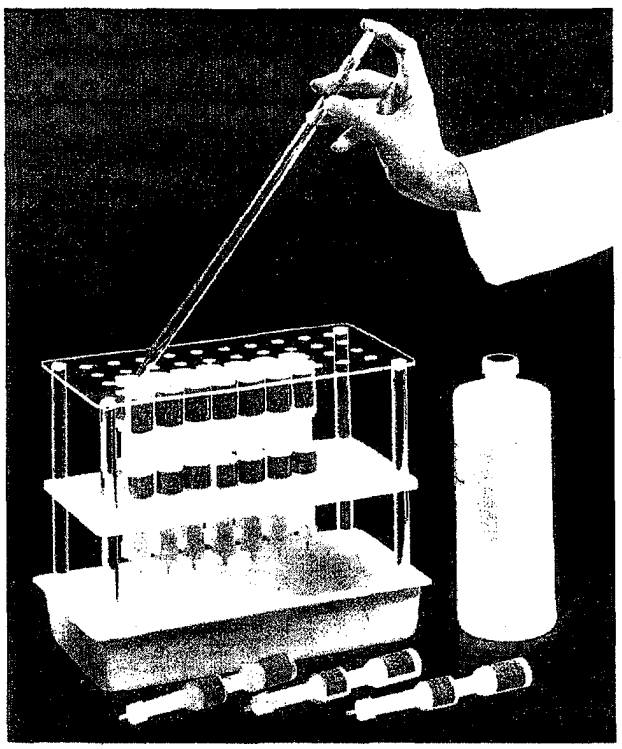
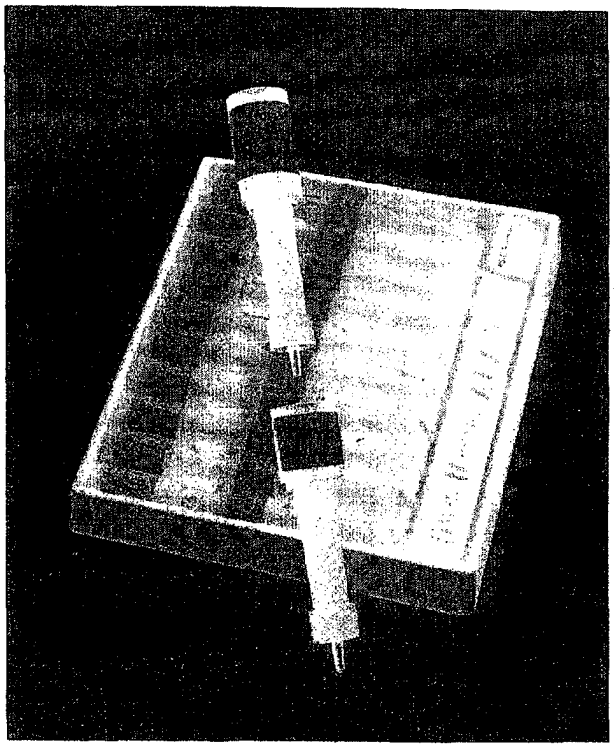
THE DETECTION OF EARLY LEAD POISONING USING THE DAVIS TEST* FOR URINARY ALA

A rapid, simplified determination of the increase in urinary delta-aminolevulinic acid (ALA) associated with excessive lead exposure . . . using the Bio-Rad prefilled, disposable ion exchange columns

* Davis, J. R. and Andelman, S. L., Arch. Environ. Health 15: 53-59 (1967)





SENSITIVE - ALA is the only urinary test that correlates closely with blood lead levels during continued lead exposure ($r > 0.9$). ALA is the only test that shows significantly elevated levels for at least one year after lead insult ceases. Only 0.5 ml of a random urine sample is required for ALA testing.

RAPID - 200 Urinary ALA tests can be run by one technician in a normal working day.

. . . AND ECONOMICAL - Urinary ALA determinations can be run at a fraction of the cost of running blood leads.

. . . AND HAS PATIENT ACCEPTANCE - Urine collection avoids the negative attitude towards venipuncture which exists in most adults and almost all children.

By far the closest correlation between clinical findings and a biochemical test (for lead poisoning) was the amount of ALA in the urine and the symptom score. Lancet.[11]

LEAD POISONING AS A PUBLIC HEALTH PROBLEM AND THE ALA TEST

The role of ALA testing in detecting industrial and general environmental lead poisoning has been clearly demonstrated. Cramer and Selander have investigated ALA testing in industry for several years[1]. They comment on its economy and accuracy and particularly on the ability of the urinary ALA test "to detect errors in the prophylactic measures at a very early stage before any symptoms have appeared." They further propose "that the ALA determination should be introduced into the regular statutory examination of lead workers, for it appears to give definite advantages in all cases."

In a survey publication from the State of California Department of Public Health, it was agreed that the concentration of blood lead in the population is now one-fourth to one-half of that recognized as hazardous to workers in industry[2]. The margin of safety for workers in battery manufacturing, printing, and lead salvage for example, is therefore, decreased by just this amount.

The composite U.S. rural exposure to lead from the air is 0.5 microgram per cubic meter and the composite urban exposure is 1.0 micrograms per cubic meter. However, several large increases from this level have been recently noted. For example, a level of 25 micrograms per cubic meter has been determined over the Los Angeles Freeways[3]. Investigators in this field recommend that ALA in urine be used as a more sensitive test than blood lead. They further propose its use in future studies of lead distribution in the population[4].

The City of Chicago has paid particular attention to pediatric lead poisoning occurring in children who have ingested lead-containing paint and plaster. The Davis test for urinary ALA was developed with the active help of the Chicago Board of Health for the anticipated screening of large numbers of children under 7 years of age living in high-incidence areas of lead poisoning[5].

The major advantage of the urinary ALA test is that it is not just another measure of lead levels in the patient's system. It is rather the earliest measure of lead induced metabolic derangement.

HISTORICAL DEVELOPMENT OF DELTA-AMINOLEVULINIC ACID (ALA) MEASUREMENT IN LEAD POISONING

1953 Shemin and Russell demonstrated that ALA was an early intermediate in porphyrin biosynthesis.[6]

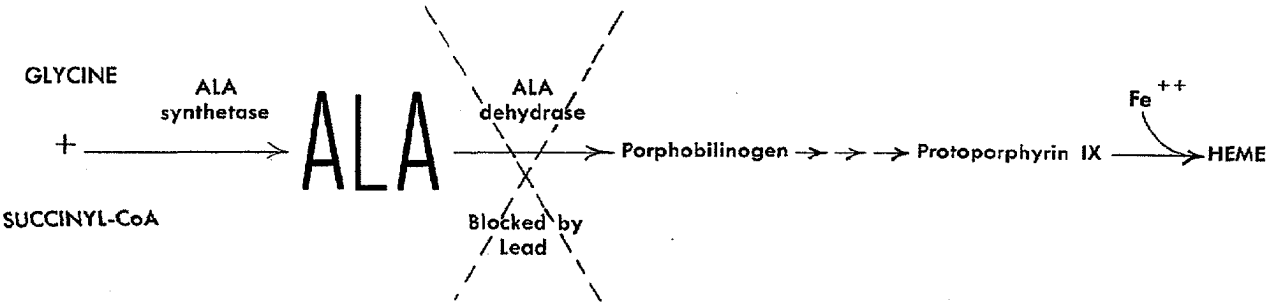
1956 Mauzerall and Granick described a method for the determination of ALA in urine employing ion-exchange chromatography for its isolation and p-dimethylaminobenzaldehyde (Ehrlich's reagent) for its colorimetric measurement.[7]

1960 Haeger-Aronsen found the concentration of urinary ALA to be increased in adult workers exposed to industrial lead environments.[8]

1965 Bonsignore, Calissano and Cartasegna suggested that the accumulation of urinary ALA occurring in experimental lead poisoning was the result of an inhibition of the enzyme ALA dehydrase.[9]

1967 Davis and Andelman modified the existing method for the determination of urinary ALA by introducing disposable, prefilled ion-exchange chromatography columns and using this technique have initiated the first large-scale investigation of urinary ALA levels in children residing in high-incidence areas of lead poisoning.[10]

MECHANISM OF DELTA-AMINOLEVULINIC ACID (ALA) ACCUMULATION IN LEAD POISONING



THE EVALUATION OF URINARY ALA AND OTHER TESTS FOR DETECTING LEAD POISONING

In a leading article, the Lancet has discussed the detection of lead poisoning. Using the clinical symptoms of the disease as the primary criteria, several laboratory tests for lead poisoning are compared.[11]

The urinary ALA test showed by far the closest correlation with the clinical picture of lead poisoning of all major laboratory tests. Lancet also points out the poor correlation of urinary coproporphyrin and also urinary lead with the same clinical criteria.

THE ADVANTAGE OF THE URINARY ALA TEST AS COMPARED WITH BLOOD LEAD MEASUREMENT IN DETECTING EARLY LEAD POISONING.

The prolonged increase of urinary ALA due to lead exposure is a major advantage of testing for urinary ALA levels rather than blood lead.

ALA levels remain increased for at least one year after lead exposure has been controlled.[12]

Blood lead levels do not exhibit this constancy since lead leaves the blood to be stored in the skeletal system. A random blood sample taken at this time can show a misleading normal lead level. When the stored lead is released, however, the dangerous effects of lead poisoning reappear.

The detection of excessive lead exposure in asymptomatic individuals can be accomplished with a random sampling technique, if the urinary ALA test is used.

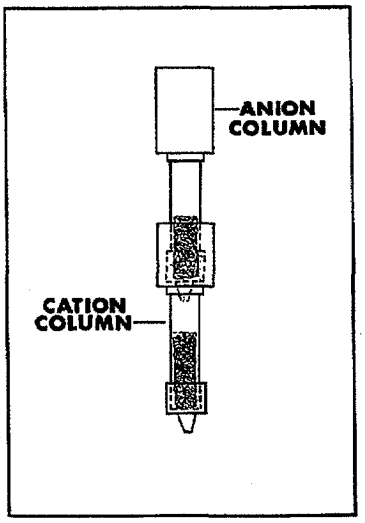
THE ADVANTAGE OF THE URINARY ALA TEST AS COMPARED WITH URINARY COPROPORPHYRIN MEASUREMENT.

Davis and Andelman tested the urine of asymptomatic children who were positive for lead poisoning by urinary coproporphyrin standards. Eighty-five percent of these coproporphyrin-positive children did not have elevated ALA levels and were thus false positive for lead poisoning.[10]

This high percentage of false positives with the coproporphyrin test has been connected with iron deficiency anemia rather than lead poisoning. Urinary ALA levels on the other hand, are not elevated by iron deficiency anemias.[13]

This lack of correlation between clinical symptoms and urinary coproporphyrin in adults and children has been noted by other investigators.

TEST PROCEDURE



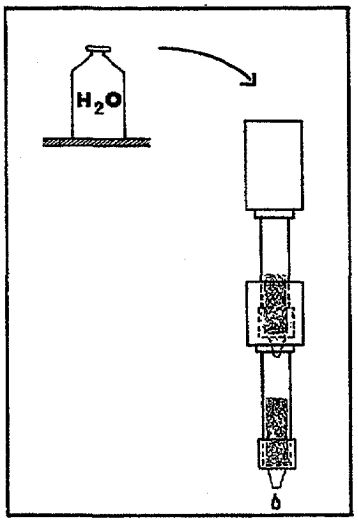
STEP 1

Technique

The top column containing anion exchange resin and the bottom column containing cation exchange resin arrive fitted together in piggyback fashion. They are placed in the drainage rack in this same position. This two column set is referred to as the column unit.

Rationale

The piggyback fitting eliminates the need to quantitively transfer the liquid passing through the top column to the bottom column.



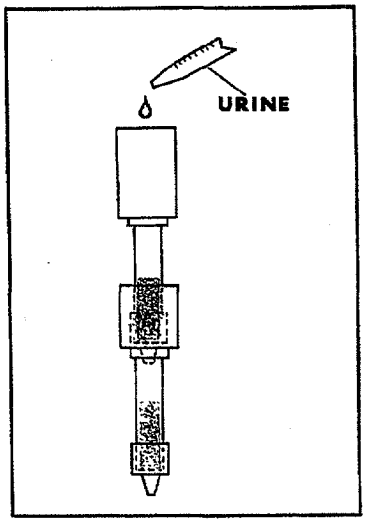
STEP 2

Technique

Apply 10 ml of distilled water to the column unit.

Rationale

Produces uniformity of column flow-rate prior to sample application.



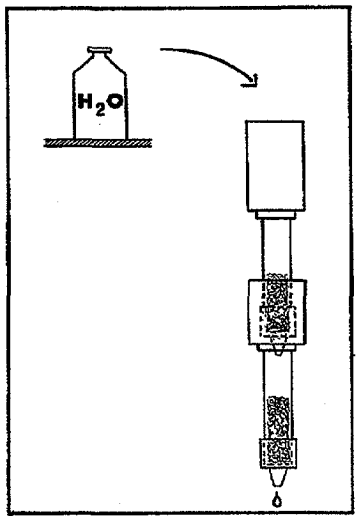
STEP 3

Technique

Apply 0.5 ml of random urine sample to the top of the column unit.

Rationale

Urine contains two substances (porphobilinogen and urea) which will interfere with the colorimetric determination of ALA. The column unit removes urea and porphobilinogen.



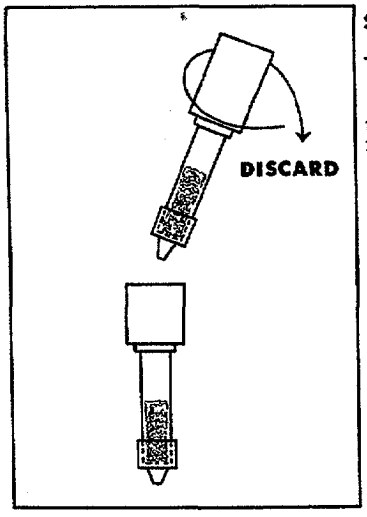
STEP 4

Technique

Add 3 aliquots of 10 ml each of distilled water to the column unit.

Rationale

Porphobilinogen is retained on top column while ALA is retained on bottom column. Urea is washed from both top and bottom columns and is therefore eliminated as an interfering substance.



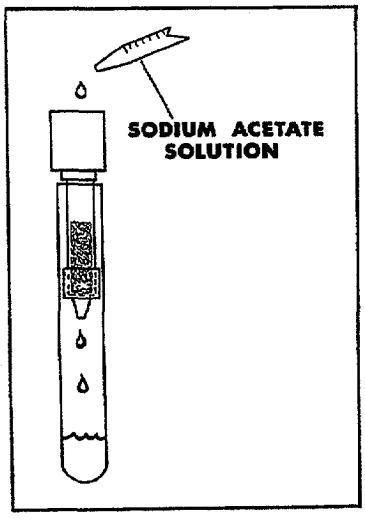
STEP 5

Technique

Remove and discard top disposable column from piggyback unit.

Rationale

Elimination of porphobilinogen retained on top column as an interfering substance.



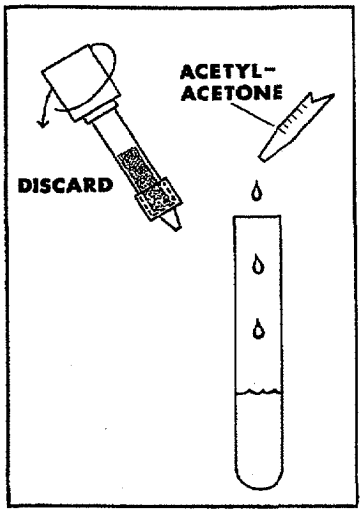
STEP 6

Technique

Place bottom disposable column in a 20 x 150 mm pyrex test tube and add 7.0 ml of 1.0 M sodium acetate to the column. Collect the drained liquid in the test tube.

Rationale

The ALA retained in the bottom column is eluted from the column with sodium acetate and the drained liquid is saved for the colorimetric analysis of ALA.



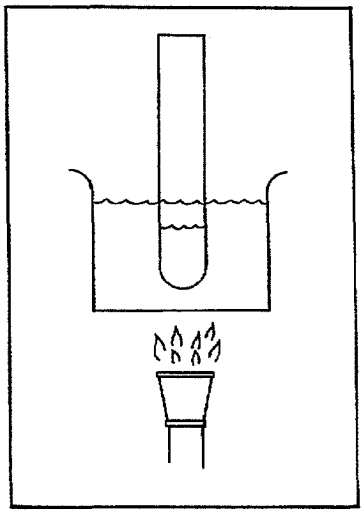
STEP 7

Technique

Remove the bottom disposable column from the test tube and discard. Add 0.2 ml of acetylacetone to the contents of the test tube and mix.

Rationale

ALA is condensed with acetylacetone to form a pyrrole.



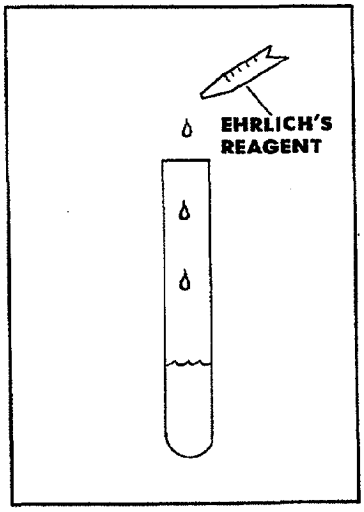
STEP 8

Technique

Place test tube in a boiling water bath for 10 minutes, remove from water bath and allow to cool to room temperature.

Rationale

A temperature of greater than 90° C allows for completion of condensation reaction to form the pyrrole in 10 minutes.



STEP 9

Technique

Add 7.0 ml of freshly prepared Ehrlich's reagent (p-dimethylaminobenzaldehyde dissolved in a mixture of acetic and perchloric acid) to test tube and mix. Allow to stand for 15 minutes.

Rationale

The pyrrole formed from ALA condenses with p-dimethylaminobenzaldehyde (DMAB) in acid solution to form a red-colored condensation product.

STEP 10

Technique

Read optical density of final mixture at 553 mμ in a standard laboratory colorimeter.

Rationale

Normal values of urinary ALA produce a faint yellow color while abnormally high values of urinary ALA produce a distinct red color. Although elevated values of urinary ALA can easily be detected by the eye, a very accurate quantitation of the urinary ALA level in terms of mg% can readily be obtained by plotting the optical density of the urine sample against known concentrations of pure ALA carried through identical procedure.

SUGGESTION FOR LARGE SCALE SCREENING

Hand-operated Continuous Syringe Type Pipetters

and/or

Automatic Pipetting Machines

have proved extremely useful for the rapid and easy delivery of all reagents used in the Davis Test for Urinary ALA employing disposable, prefilled ion-exchange chromatography columns.

COMPARISON OF PRE-TEST SAMPLE COLLECTION METHODS

URINARY ALA

1. Only 0.5 ml of a random urine sample is required for the determination of ALA, allowing for ease of sample collection especially from the younger age group. Urine samples can be stored in a dark freezer for several months without loss of the original ALA content.

2. Sample collection can be accomplished by non-medical personnel in a non-hospital environment, allowing for direct sample collection at the home or place of employment. The problem involved using collection containers possibly contaminated with traces of lead does not exist for the urinary ALA test.

3. No pain or trauma is associated with urine sample collection.

BLOOD LEAD

1. A sample size of 5-10 ml of whole blood is required, this amount being quite difficult to obtain by cubital venipuncture in a small child or infant.

2. Sample collection must be performed by a physician or a highly-trained medical technician, often requiring the aid of a second person when dealing with a small child.

3. Pain and trauma are often associated with blood sample collection, resulting in wide spread adult patient resistance to the test as well as marked parent resistance to the test when dealing with asymptomatic, apparently-well small children.

THE DAVIS TEST FOR URINARY ALA

The measurement of Delta-Aminolevulinic Acid (ALA) colorimetrically depends upon the preliminary removal of urea and porphobilinogen from the urine. Traditionally this has required the laboratory preparation of two separate ion exchange columns for each determination.

Using specially processed resins in piggy-back columns, the Davis Test gives the laboratory a prepared disposable unit ready for use with simple reagents.

With this test now available, screening for excessive lead exposure and early lead poisoning can be accomplished with great reliability and economy.

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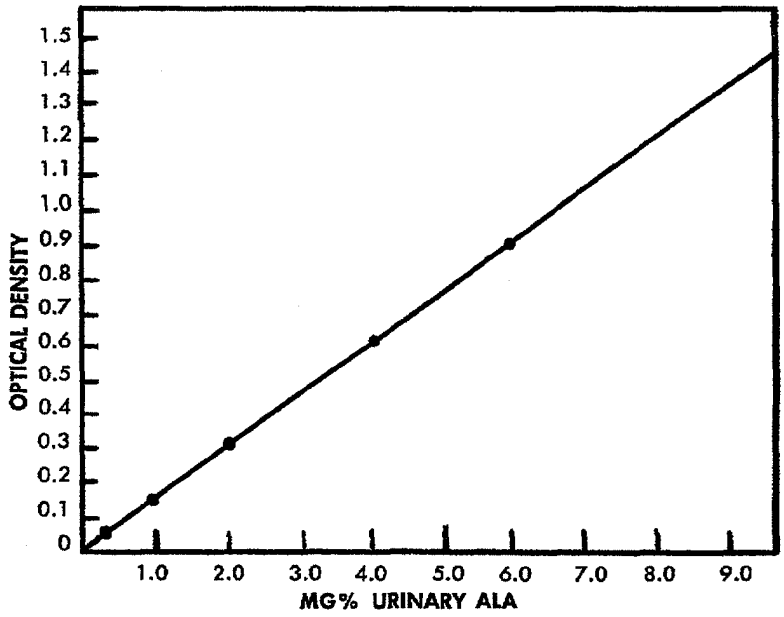
PREPARATION OF A STANDARD CALIBRATION CURVE

1. Six aliquots of the ALA hydrochloride stock solution (0.01 ml, 0.05 ml, 0.10 ml, 0.20 ml, 0.30 ml and 0.50 ml) are used as working standards.

Volume of standard stock solution (1 ml = 100 µg)	µg of ALA per total reaction volume	Corresponding mg% of urinary ALA for a 0.5 ml sample of urine applied to the column
0.01 ml	1 µg	0.20 mg%
0.05 ml	5 µg	1.00 mg%
0.10 ml	10 µg	2.00 mg%
0.20 ml	20 µg	4.00 mg%
0.30 ml	30 µg	6.00 mg%
0.50 ml	50 µg	10.00 mg%

2. Dilute each of the six working standards to 7.0 ml mark with 1.0 M sodium acetate using graduated test tubes.
3. Pipet 7.0 ml of 1.0 M sodium acetate into an empty test tube to be used as a reagent blank.
4. Add 0.2 ml of acetylacetone to each tube and mix.
5. Place tubes in a boiling water bath for 10 minutes, remove and cool to room temperature.
6. Add 7.0 ml of Ehrlich's reagent to each tube, mix and let stand for 15 minutes for complete color development.
7. Set a colorimeter to 100% transmission at 553 mµ using the reagent blank.
8. Read the optical density of the standards and unknowns.
9. Prepare a standard curve by plotting the concentrations of the standards against optical density.
10. Read the concentration of urinary ALA in the unknowns (0.5 ml urine sample applied to the column unit) in terms of mg% from the sample calibration curve shown below:



INTERPRETATION OF URINARY ALA VALUES

URINARY ALA RANGE (mg %)	URINARY ALA CODE
0.00 - 0.54	NORMAL
0.55 - 0.99	TRACE
1.00 - 1.49	1 PLUS
1.50 - 1.99	2 PLUS
2.00 - 2.99	3 PLUS
3.00 - 5.99	4 PLUS
6.00 -10.00	5 PLUS

AVERAGE COMPLETION TIME FOR ALA DETERMINATION

20 MEASUREMENTS REQUIRE 1 HOUR

40 MEASUREMENTS REQUIRE 2 HOURS

80 MEASUREMENTS REQUIRE 3 HOURS

200 MEASUREMENTS REQUIRE 5 HOURS

ORDERING INFORMATION

Catalog No.	Description	Pricing
TEST KITS		
95002	20 Piggy-back Column Units (Complete with reagents* and standard)	$ 34.50
95004	40 Piggy-back Column Units (Complete with reagents* and standard)	60.00
95020	200 Piggy-back Column Units (Complete with reagents* and standard)	207.00
95501	* Note: Perchloric acid (72%) is not supplied with the kit but is available in one pound bottles sufficient for 200 tests.	4.00
SUPPORT RACK FOR HOLDING DISPOSABLE COLUMNS DURING TEST PROCEDURES		
95502	Support rack for 40 Column Units with drain tray and numbered pipet guide as shown on page 1.	13.75
COLUMNS ONLY		
95108	80 Piggy-back Column Units	94.00
95120	200 Piggy-back Column Units	190.00
Prices will be quoted on larger amounts.		
REPIPETS® FOR EXTRA CONVENIENCE		
95801	1 ml, with 950 ml amber bottle	47.50
95802	10 ml, with 950 ml amber bottle	47.50
All prices are FOB shipping point, either Richmond, California, or New York City. Terms: Net 30 days.		

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