STEROID-SKREEN

Urinary 17-Hydroxy Corticosteroids

(NON-HYDROLYTIC)

BIOCHEMICAL
DIAGNOSTICS, INC.

PRECAUTIONS: For in vitro diagnostic use only. Please read the entire insert and carefully follow handling and stability instructions before performing tests.

There may be slight variations in the composition of different lot numbers of a particular reagent. Reagents from different lot numbers should not be used separately in a single run. They should be 'pooled' and used as a homogenous reagent, i.e., a sufficient amount of 'color reagent' should be reconstituted and pooled for each run. For best results this rule should also be applied to reagents of the same lot number.

SUMMARY

When complete, the Steroid-Skreen kit contains reagents for the quantitative determination of 17-OH corticosteroids in unine. Ample reagents are supplied for 50 or 500 tests.

PRINCIPLE

The method involves column adsorption of the 17-OH corticosteroids (conjugated and "free") from 10 ml of a 24 hour non-hydrolyzed urine collection. After simple column washes, the corticosteroids are eluted and measured by the Porter-Silber method. This procedure eliminates liquid/liquid extractions and the subsequent drying down of elution solvents. 27 samples can be completed in about 2½ hours. Technician time is less than 45 minutes.

CONTENTS OF 17-OH CORTICOSTEROIDS KIT

Catalog No.	Description	
35 06 261-1	17-OH Corticosteroid Kit, 50 determinations (without reusable sample reservoirs), consisting of:	
	4 vials	Phenylhydrazine, 40 mg
	1 vial	Hydrocortisone, 10 mg
	1 container	Sodium Chloride, 120 g
	1 bottle	Elution Solvent, 80% ethanol, ½ liter
	1 bottle	Saline, pH 7.0, 250 ml
	1 bottle	Ethanol, 5%, ½ liter
	1 bottle	Reagent Blank, 75% sulfuric acid, 325 ml
	1 bottle	Ethanol, anhydrous, 25 ml
	1 bottle	Sodium Hydroxide, 2N, 60 ml
	50	Clini-Skreen Columns
	1 bottle	Column Activator "A" Concentrate, 40 ml
	3	Sodium Chloride Measuring Thimbles, 2.2 g
35 06 094-3	As above, but without hydrocortisone, Clini-Skreen Columns, and Column Activator "A" Concentrate	

STEROID-SKREEN BULK REAGENT PACKS*

Catalog No.	Description	
35 06 303-0	Urinary 17-OH Corticosteroids, without hydrolysis, dry portion, 500 determinations, consisting of:	
	3 vials	Hydrocortisone, 10 mg
	1 container	Sodium Chloride, 1.11 kg
	40 vials	Phenylhydrazine, 40 mg
	5	Sodium Chloride Measuring Thimbles, 2.2 g
35 06 301-3	Urinary 17-OH Corticosteroids, without hydrolysis, liquid portion, 500 determinations, consisting of:	
	1 container	Reagent Blank, 3.1 liters
	1 Cubitainer®	Elution Solvent, 5 liters
	1 bottle	Sodium Hydroxide, 2N, ½ liter
	1 Cubitainer	Saline, pH 7.0, 2.5 liters
	1 Cubitainer	Ethanol, 5% 5 liters

*500 Clini-Skreen Columns, 400 ml Column Activator "A" Concentrate and 30 ml anhydrous ethanol (see #3 under "NOTES AND PRECAUTIONS") are required but not supplied with bulk reagent packs.

Cubitainer® is a registered trademark of the Hedwin Corporation.

REAGENTS

Precautions, Stability and Preparation

PHENYLHYDRAZINE (Color Reagent)

Precautions WARNING: Hazardous material. Avoid contact with skin. Do not pipet by mouth. Contact physician if ingested.

Stability Stable for at least one year when stored in the dark at 2-8°C

PHENYLHYDRAZINE (continued)

Preparation Reconstitute each vial (as needed) with 40 ml reagent blank. The reconstituted color reagent is stable for one week when stored in the dark at 2-8°C.

HYDROCORTISONE

Precautions CAUTION: Avoid contact with skin. Do not pipet by mouth.

Stability Stable for at least two years when stored in the dark at 2-8°C.

Preparation Reconstitute each vial with 10 ml anhydrous ethanol. The reconstituted standard is stable for at least 3 months when stored in the dark at 2-8°C (or 6 months at −20°C).

ELUTION SOLVENT, ANHYDROUS ETHANOL AND 5% ETHANOL

Precautions CAUTION: Poison—denatured with 95% SDA-3A and 5% isopropanol (see #3 under "NOTES AND PRECAUTIONS"). Keep away from heat, sparks and open flame. Do not pipet by mouth.

Stability Stable for at least two years at room temperature.*

SODIUM HYDROXIDE, 2N

Precautions CAUTION: Caustic reagent; avoid contact with skin, eyes and clothing. Do not pipet by mouth.

Stability Stable for at least two years when stored at room temperature.

*Room temperature is defined as 15-30°C.

SODIUM HYDROXIDE, 0.1N

Precautions CAUTION: Caustic reagent; avoid contact with skin, eyes and clothing. Do not pipet by mouth.

Stability Unstable. Use within 24 hours.

Preparation Add 5 ml 2N sodium hydroxide to 95 ml distilled water.

REAGENT BLANK

Precautions CAUTION: Corrosive reagent; avoid contact with skin, eyes and clothing. Do not pipet by mouth.

Stability Stable for two years when stored at room temperature in a tightly closed container.

SALINE, pH 7.0

Precautions For in vitro diagnostic use only.

Stability Stable for at least two years when stored at room temperature.

SODIUM CHLORIDE

Precautions For in vitro diagnostic use only.

Stability Stable for at least two years when stored at room temperature.

AMBERLITE XAD-2® CLINI-SKREEN COLUMNS

Precautions The Clini-Skreen Column contains Amberlite XAD-2® resin which is impregnated with a relatively non-volatile wetting agent. The impregnated resin remains in an activated state when stored at less than 25°C.

AMBERLITE XAD-2® CLINI-SKREEN COLUMNS (continued)

To compensate for any gradual loss or contamination of the wetting agent which might affect adsorption, the column should be washed with the 7% Column Activator "A" Solution prior to sample addition. (See "Column Preparation.")

Stability Stable until date stamped on package when stored at less than 25°C and sealed tightly after each use.

COLUMN ACTIVATOR "A" CONCENTRATE

Precautions Poison: Keep away from heat, sparks, and open flame. Do not pipet by mouth.

Stability The 7% solution and concentrate are stable for two years when stored at room temperature.

Preparation For each 100 ml of the 7% Column Activator "A" Solution required, pipet 7 ml of the Activator "A" Concentrate into a measuring cylinder and bring to 100 ml with distilled or deionized water. 10 ml of this solution are required for each column used.

ADDITIONAL ITEMS

The following items are not supplied with Steroid-Skreen Kits, but are necessary for the determination of urinary 17-OH corticosteroids.

Water bath (60°C)

Spectrophotometer or colorimeter with a wavelength of 410 nm

Vortex mixer

PROCEDURE

MATERIALS PROVIDED

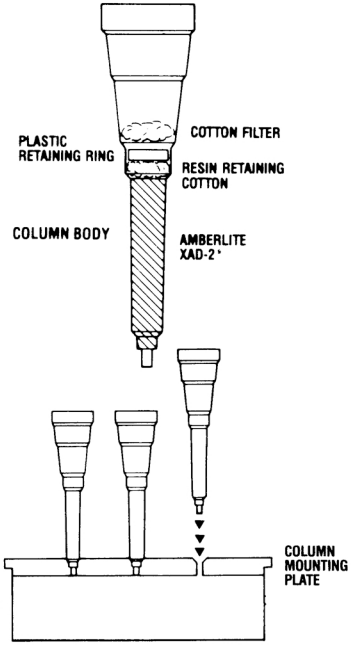
The materials provided with this kit are those listed under "CONTENTS".

SAMPLE COLLECTION

Collect a 24 hour urine sample (HCl or boric acid may be used as a preservative.) Urine may be stored at 2-8°C for 72 hours or frozen at −25°C for longer periods.

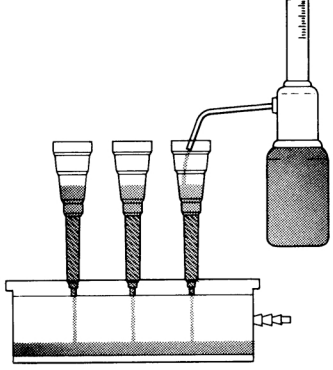
Amberlite XAD-2® is a registered trademark of the Rohm & Haas Co.

COLUMN PREPARATION



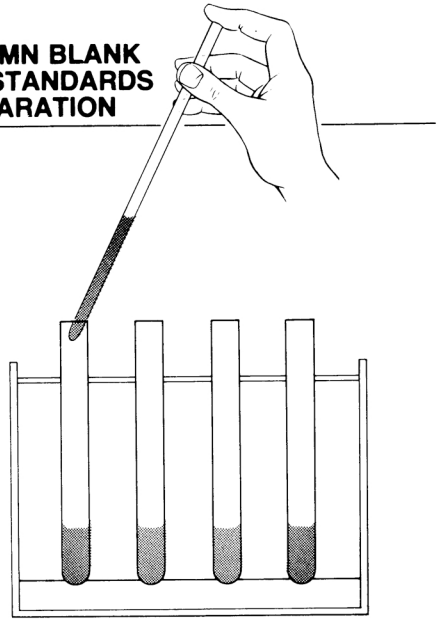
1. Place prepared columns in column mounting plate, located on the Aspirator Trough.

2. Check that the cotton filter is loosely seated against the plastic retaining ring.



3. Wash each column with 10 ml of a 7% solution of Column Activator "A" Concentrate.

COLUMN BLANK AND STANDARDS PREPARATION

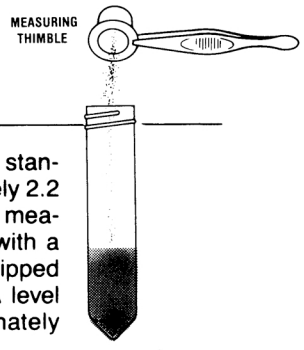


1. Pipet 10 ml saline pH 7.0 into a disposable test tube for the column blank.

2. Use microliter pipettes to add 0.05, 0.10, 0.20 and 0.25 ml of the stock standard into separate tubes each containing 10 ml saline pH 7.0 and mix.

SAMPLE PREPARATION

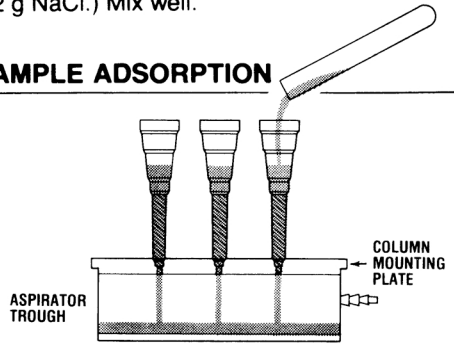
Adjust a 10 ml urine aliquot to pH 6–8.



SALT ADDITION

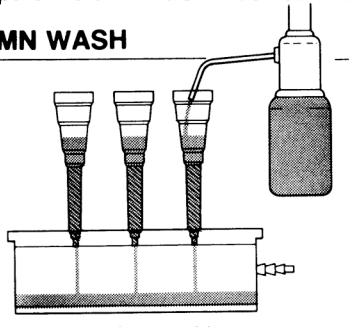
Add to each urine sample, standard and blank approximately 2.2 g of NaCl. (The convenient measuring thimble is grasped with a tweezer or hemostat and dipped into a container of NaCl. A level thimble contains approximately 2.2 g NaCl.) Mix well.

SAMPLE ADSORPTION



Pour the prepared column blank, standards and urines into separate columns and allow to drain by gravity. Sample should flow at approximately 3 ml/minute. While the columns are draining, prepare the 0.1N NaOH wash solution.

COLUMN WASH

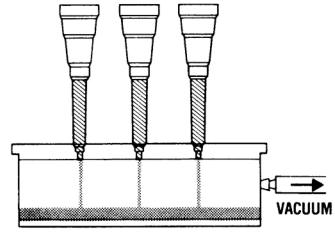


Wash each column with:

1. 10 ml 0.1N NaOH. (Freshly prepared)
2. 10 ml deionized water.
3. 10 ml 5% ethanol.

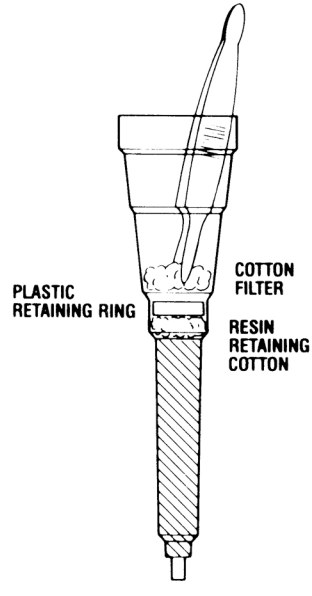
Allow all washes to drain through the column by gravity. Wash solutions should flow at approximately 2 ml/minute.

COLUMN ASPIRATION*



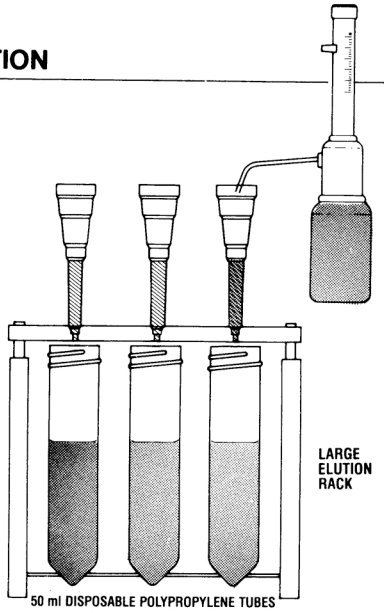
1. Aspirate excess solvent by attaching a water aspirator to the lower end of the column for 30 seconds or simultaneously aspirating columns with the Clini-Skreen apparatus for at least three minutes (using a vacuum pump with trap). Check for proper aspiration by placing hand on top of reservoir. A vacuum pull should be felt.

*A good aspiration is essential to the procedure. Interfering chromogens may remain in unremoved washing solution and subsequently be eluted with the 17-OH corticosteroids.

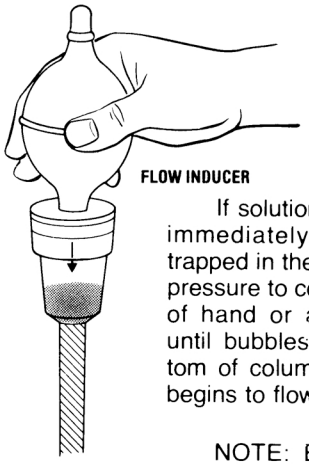


2. During the column aspiration step, remove the cotton filter leaving the retaining cotton plug in place below the plastic retaining ring.

ELUTION



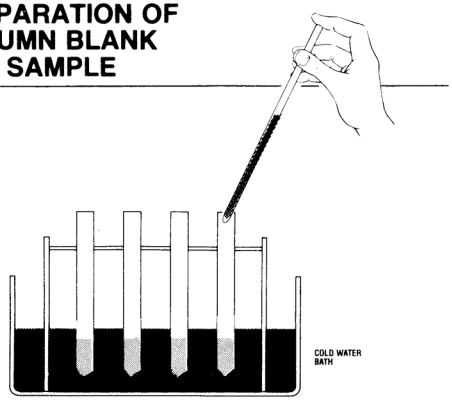
Place column mounting plate on large tube rack and elute each column with 10 ml elution solvent. Collect in 50 ml tube. *Mix thoroughly.* While the columns are draining prepare the phenylhydrazine color reagent.



If solution does not flow immediately, air may be trapped in the column. Apply pressure to column with heel of hand or a flow inducer until bubbles cease at bottom of column and solution begins to flow.

NOTE: Elution solvent should flow at approximately 2 ml/minute.

COLORIMETRIC PREPARATION OF COLUMN BLANK AND SAMPLE



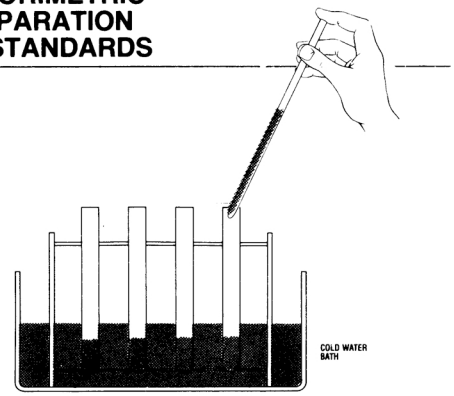
1. Two aliquots of 2 ml each of the column eluates are transferred into separate test tubes.

2. Place all tubes in cold water bath to prevent overheating.

3. One aliquot is mixed with 3 ml reagent blank and one aliquot is mixed with 3 ml color reagent.

Mix on a vortex mixer for 15 seconds. Complete mixing is essential.

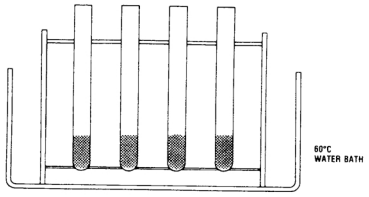
COLORIMETRIC PREPARATION OF STANDARDS



1. Transfer only one aliquot of 2 ml from each standard eluate into test tubes which have been placed in a cold water bath.

2. Add 3 ml color reagent to each solution and mix on a vortex mixer for 15 seconds. Complete mixing is essential.

COLORIMETRIC INCUBATION



1. Place all tubes in a water bath at 60°C for 30 minutes.
2. Following incubation, place all tubes in a cold water bath for approximately 5 minutes to bring to room temperature.

COLORIMETRIC ASSAY

1. Mix all tubes for 10 seconds on a vortex mixer. Complete mixing is essential.
2. Samples and standards are read at 410 nm after first zeroing instrument against the column blank aliquot containing color reagent.
3. Sample blanks are read at 410 nm after first zeroing instrument against the column blank aliquot containing reagent blank.

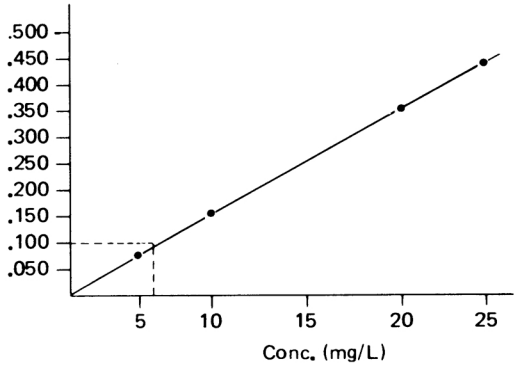
CALCULATIONS

1. Sample blank readings are subtracted from sample readings.
2. The results of the standards are used to plot an absorbance/concentration curve.
3. The sample concentration is determined from this curve and multiplied by the total 24 hour specimen volume and divided by 1000. The figure obtained represents the total amount of 17-OH corticosteroids excreted during the collection period.

SAMPLE CALCULATION

Based on a 24 hour urine collection of 1500 ml.

Concentration	Test Blank	Corr. A
5 mg/L std.	.095	.095
10 mg/L std.	.190	.190
20 mg/L std.	.380	.380
25 mg/L std.	.475	.475
Unknown	.257, .157	.100



C_{unk} (mg/L) = 5.3

$$C_{unk}\ (mg/24\ hr) = \frac{C_{unk}\ (mg/L) \times T.V.}{1000}$$

$$= \frac{5.3 \times 1500}{1000} = 8.0\ mg/24\ hr$$

A = Absorbance
C = Concentration
C_{unk} = Concentration of the unknown
T.V. = Total Volume of the 24 hour urine collection
Corr. A = Corrected absorbance

These are sample calculations only and are not to be used in calculating the results. After the color reaction, samples should be read within one hour.

QUALITY CONTROL

It is recommended that a commercially available urine control be run as a check on the procedure.

Bio-Rad Laboratories list our procedures on their assay sheets, and their controls have been assayed by our Quality Control Section.

LIMITATIONS

This Steroid-Skreen procedure is designed for the quantitative determination of 17-OH corticosteroids in urine only. The user should be aware that certain drugs are known to affect the Porter-Silber reaction. Clini-Skreen Newsletter No. 3, January, 1976, lists compounds that have been reported to interfere with these tests in previously reported liquid/liquid procedures.

NOTES AND PRECAUTIONS

1. Test tubes should be disposable, if possible. (Polypropylene tubes are recommended.)

If test tubes must be rewashed, use a phospate-free detergent and rinse well with distilled water. Do not dry with acetone. Contaminated glassware is the most common source of error in this procedure.

2. All incubations must be done in a water bath. An incubation oven provides insufficient heat transfer.

3. Two types of ethanol may be used for reagents and elution:

a. Absolute (undenatured)

b. Anhydrous (denatured with SDA 3-A and 5% isopropanol).

Denaturing formulas containing ketones and aldehydes cannot be used, since they adversely affect color development.

4. Follow the instructions carefully.

NORMAL RANGE

17-OH CORTICOSTEROIDS

Adult Male: 3–10 mg/24 hours

Adult Female: 2–6 mg/24 hours

Each laboratory should establish a normal range for the particular population it is studying. The range given has been established from in-patient and out-patient hospital subjects.

PERFORMANCE CHARACTERISTICS

Precision

1. Multiple determinations within a single day:
   n = 10
   mean = 4.6 mg/24 hours
   standard deviation = 0.3
   coefficient of variation = 6.5
2. Day to day variation:
   n = 15
   mean = 12.0 mg/24 hours
   standard deviation = 0.4
   coefficient of variation = 3.3

RECOVERY

Varying amounts of hydrocortisone and tetrahydrocortisone glucuronide were added to urine specimens and carried through the entire procedure:

n = 15
mean % recovery = 87.4%
standard deviation = 3.5
coefficient of variation = 4.0

CORRELATION

Steroid-Skreen (Non-Hydrolytic, mg/24 hours)	Steroid-Skreen (Enzymatic, mg/24 hours)	Liquid/Liquid Extraction (Enzymatic[1], mg/24 hours)
2.7	2.3	2.6
16.4	15.8	16.7
7.2	6.9	6.5
7.8	7.8	7.6
9.3	8.8	10.6
5.2	5.0	5.8
7.4	7.4	7.0
6.7	6.3	6.3

[1]Kream, J., et al: Advances in Automated Analysis. Technicon International Congress, 1970. p. 555.

BIBLIOGRAPHY

1. Drekter, I.G. et al.: J. Clin. Endocrin. & Metab. 12:55, 1952.
2. Metcalf, M.G.: A rapid method for measuring 17-OH corticosteroids in urine. J. Endocrin. 26:415–423, 1963.
3. Chu, T.M., Osawa, Y., and Reynoso, G.: Simple assay for urinary estrogens in nonpregnancy. Clin. Chem. 17:438, 1971.
4. Osawa, Y., and Slaunwhite, W.R.: Studies on phenolic steroids in human subjects. XIII. A rapid assay of urinary estrogen conjugates in pregnancy. Steroids 15:73, 1970.
5. Shackleton, C.L., Sjovall, J. and Wilson, D.: A simple method for assay of urinary estriol in pregnancy. Clin. Chem. 16:532, 1970.
6. Bradlow, H.L.: Extraction of steroid conjugates with neutral resin. Steroids 11:265, 1968.
7. Lee, L., and Haknel, R.: Rapid fluorometric assay of estrogen conjugates in pregnancy urine. Clin. Chem. 17:1104, 1971.
8. Wiedermann, M., Raith, L., Wirtz, A., and Karl, H.J.: Separation of free and protein-bound steroids with Amberlite. Z. Anal. Chemie 261:382, 1972.
9. Levy, S.: Clin. Chim. Acta, to be published.
10. Levy, S. and Schwartz, T.: A simple colorimetric method for the extraction and determination of urinary 17-ketosteroids using styrene divinylbenzene copolymer XAD-2 resin columns. Clin. Chem. 19:679, 1973.
11. Haknel, R., and Jones, H.: Dichloromethane for the extraction of the color in the Kober Reaction according to Bradshaw. Clin. Chim. Acta 16:185, 1967.
12. Backloff, S., Faucette, W. and Cowley, L.P.: A rapid method for assays of urinary estriol in pregnancy.

OTHER QUALITY CLINICAL PRODUCTS INCLUDE:

VMA-Skreen Reagent Kits

17-Ketosteroid Reagent Kits

17-OH Corticosteroid Reagent Kits