SCOPESCREEN

1/2/17 Revised 01/11/2020 (Formerly known as BIOSCREEN)

CITRICSCREEN®

Photometric Method for Detecting Citric Acid in Seminal Plasma

(96 determinations)

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

Principle:

CitricScreen® measures the amount of citric acid in seminal plasma.

In the *first step*, semen is treated with alcohol and then centrifuged so as to remove spermatozoa and particulates from the seminal plasma.

In the *second step*, a solution of ferric chloride is added to a known amount of seminal plasma. Any citrate present will cause the solution to turn yellow. The intensity of color can be measured in a microplate reader and is directly related to the amount of citrate ions in the seminal plasma. Using a formula, the total amount of citric acid present can be calculated.

Reagents:

Ferric Chloride: 15 ml of ferric chloride solution. Ready to use.
Alcohol: 10 ml alcohol. Ready to use.
Citric Acid Standard: 1.5 ml of 2 mg/ml citric acid solution. Ready to use. *Caution:* Avoid contact with skin and eyes.
Plate: 96 wells.

Materials Required But Not Provided:

1. Distilled or deionized water.
2. Centrifuge, capable of 1500*g*.
3. Test tubes and rack.
4. Micropipettors and tips.
5. Collecting cups.
6. Microplate reader, capable of reading at 390 nm to 410 nm.

Storage and Stability:

Store the reagents at room temperature. They can be used until the date shown on each reagent label. The expiration date is 18 months from the date of manufacture.

Warning and Precautions:

All semen samples should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Specimens should be disposed of in accordance with OSHA guidelines.

Specimen Collection:

Semen should be collected in a clean cup. The semen sample should be stored at room temperature until using. Or, semen can be stored frozen by placing specimen in an ordinary freezer until assaying at a later time. No special procedures are needed for freezing and thawing.

Procedure:

1. Allow semen sample to liquefy and measure total volume of semen.
2. Pipette 100 µl semen sample into a test tube.
3. Add 100 µl **Alcohol** to the test tube and mix.
4. Centrifuge for 10 minutes at 1500*g*.
5. Pipette 50 µl supernatant into an empty well.
6. Pipette 50 µl **Citric Acid Standard** into an empty well.
7. Pipette 50 µl water, as a negative control, into an empty well.
8. Add exactly 5 drops **Ferric Chloride** to each well.
9. Briefly agitate **Plate** to mix contents.
10. Read optical density (OD) of wells at 390 nm (or up to 410 nm) using a microplate reader. First adjust the microplate reader to zero while reading the negative control and then read the other wells.

NOTE: The OD reading of the Citric Acid Standard should fall between 0.10 and 0.25. Repeat the test if your Citric Acid Standard is lower (<0.1) or higher (>0.25)

Calculation of Total Citric Acid:

$$\text{Total Citric Acid} = \frac{OD_{\text{seminal plasma}}}{OD_{\text{Citric Acid Standard}}} \times 4 \times \text{Volume}$$

where 4 is a constant determined by the amount of citric acid in the **Citric Acid Standard** and the dilution of the specimen.

Example: At 410 nm the following were obtained for a semen specmen:
Semen Volume = 2.0 ml
Mean OD of Specimen = 0.23
Mean OD of Citric Acid Standard = 0.14
Applying the formula: $\frac{0.23}{0.14} \times 4 \times 2 = 13$ mg citric acid/ejaculate

Performance Characteristics:

Intra-Assay Reproducibility

A semen specimen was assayed 13 times for citric acid:

Number	Mean (mg)	S.D.	C.V.
13	6.8	± 0.40	6%

Spiking Recovery

A semen pool was spiked with 0, 1, 2, and 4 mg citric acid and then tested by the **CitricScreen®** method. The observed results were determined using a standard curve of citric acid.

Spike (mg)	O Observed (mg)	E Expected (mg)	O/E Recovery (%)
0	0	--	--
1	1.3	1.0	130
2	2.0	2.0	100
4	4.0	4.0	100

Selected References:

Sharma N, Acharya A, Singh SK, Singh M, Sharma U, Prasad R. Heterogenous spectrum of CFTR gene mutations in Indian patients with congenital absence of vas deferens. Hum Reprod 2009;24:1229-1136.

World Health Organization. *Laboratory manual for the examination of human semen and sperm-cervical mucus interaction*. 3rd ed. New York: Cambridge University Press, 1992.

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