

# Citric Acid Test – 96 Tests

IVD

PHOTOMETRIC TEST FOR QUANTIFYING CITRIC ACID IN HUMAN SEMINAL PLASMA

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Reagent for professional use only

## GENERAL INFORMATION

The secretory products of the prostate gland form about one-third of the seminal fluid in which spermatozoa are suspended. The level of Citric Acid in semen gives a reliable measure of prostate gland secretion<sup>1</sup>. It has been speculated that citric acid may be important in maintaining the osmotic equilibrium of semen; this, in turn will affect the membrane function and morphology of the spermatozoa.

The Citric Acid Test may help in assessing the diagnosis and the management of male infertility.

## TEST PRINCIPLE

The Citric Acid test works in two steps:

1. Spermatozoa and particles are removed by isopropanol (causes precipitation)
2. After centrifugation, ferric chloride is added to the supernatant. The Fe<sup>3+</sup>-ions and citrate form a complex that turns the solution to a yellow colour. The intensity of the colour is directly related to the amount of citrate and can be measured in a photometer or plate reader.

## MATERIAL INCLUDED IN THE KIT

Reagent 1 - 20ml FeCl<sub>3</sub> solution (Foams easily: Do not shake)

Reagent 2 - 10ml Isopropanol

Reagent 3 - 2ml Citric Acid standard (4mg/ml)

A certificate of analysis and MSDS are available upon request or can be downloaded from the website.

## MATERIAL NOT INCLUDED IN THE KIT

- Plate reader / Photometer (405nm filter)
- Pipettes and tips
- Centrifuge tubes
- Titre plate
- Centrifuge (≥2500g)
- Eppendorf tubes

## SPECIMEN TYPE

The test is preferably done on (frozen/thawed) semen plasma instead of the whole semen sample. This, to avoid the formation of excessive precipitation during the analysis. Freeze seminal plasma if the sample cannot be tested at the same day of ejaculation.

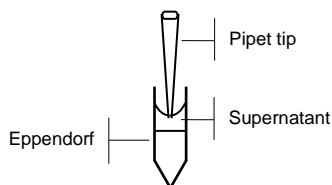
## METHOD

We recommend to view our demonstration video (download via link on our website, or scan barcode e.g. with the App "REA PharmaScan"):



1. Add 100µl of Reagent 2 to 100µl of semen or semen plasma and mix gently.
2. Standard: gently mix 100µl of standard solution with 100µl of Reagent 2
3. Centrifuge for 20 minutes at 2500g
4. Pipette 25µl of supernatant into an empty well as follows:
  - Place pipet tip in the middle of the Eppendorf tube, right below the meniscus of the supernatant
  - Slowly move pipet tip downwards whilst aspirating fluid

**Note:** Remove supernatant very carefully in order not to aspire any of the sediment. In case the supernatant is turbid, centrifuge again at 2500g for another 10 minutes



5. Add 200µl of Reagent 1 to the well, slowly. Mix gently to avoid air bubbles.
6. Read the results of the samples and the standard at 405nm

## CALCULATION OF RESULTS

Divide the measured OD value for the sample by the OD from the standard, and multiply by the concentration of the standard (4mg/ml):

$$\text{Citric Acid mg/ml} = \frac{\text{OD sample}}{\text{OD standard}} \times 4\text{mg/ml}$$

To obtain total citric acid amounts, multiply the result with the total volume of the semen sample or seminal plasma. Normal citric acid value is 10mg or more per ejaculate<sup>2</sup>.

A reduction in citric acid was detected in patients with prostatitis and in patients with azoospermia<sup>1</sup>. Kanyo (1975)<sup>3</sup> concluded that low values of citric acid in the ejaculate support the urological diagnosis of chronic prostatitis or hypo-gonadism.

## ASSAY PERFORMANCE PARAMETERS

Intra-assay CV: 4% (Repeatability)

Inter-assay CV: 8% (Total precision)

## STORAGE

Suitable for transport or short term storage at elevated temperatures (up to 5 days at 37°C). Store reagents between 2°C and 25°C. Keep away from light. Product can be used for 12 months after date of production.

## WARNINGS AND PRECAUTIONS

Reagent 1: Harmful if swallowed. Irritating to skin. Risk of serious damage to eyes.

Reagent 2/3: Highly flammable. Irritating to eyes. Vapours may cause drowsiness or dizziness.

All human, organic material should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens and reagent (gloves, lab vest, eye/face protection).

## CALCULATION OF G-FORCES

The g-force of your centrifuge can be calculated using this formula:

$$g = 1.118 \times r \times \text{rpm}^2 \quad \text{or} \quad \text{rpm} = \text{Square root} \{g / (1.118 \times r)\}$$

r = radius of centrifuge in mm

rpm = rotations per minute / 1000

e.g.1

$$r = 100 \text{ mm} \quad \text{rpm} = 3000 \text{ rotations per minute}$$

$$g = 1.118 \times 100 \times 9 = \underline{1006g}$$

e.g.2

$$r = 100 \text{ mm} \quad g = 1200g$$

$$\text{rpm} = \text{SQR} \{1200 / (1.118 \times 100)\} = 3.28$$

$$= \underline{3280} \text{ rotations per minute}$$

## BIBLIOGRAPHY

1. F.H. Comhaire; Male Infertility. Clinical investigation, cause evaluation and treatment - 1st Edition; Chapman & Hall; 1986
2. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction, 3rd Edition, Cambridge Press, 1992
3. Kanyo A., Sas M., Citric Acid Contents in the Ejaculate. Significance of Its Determination in Andrological Diagnostics, International Urology and Nephrology, 1975, 7(1), pp.83-7

## TECHNICAL SUPPORT



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