EPISCREEN PLUS™

NEUTRAL ALPHA-GLUCOSIDASE ASSAY (25 TESTS) - IN VITRO DIAGNOSTIC DEVICE FOR THE QUANTITATIVE MEASUREMENT OF NEUTRAL ALPHA-GLUCOSIDASE IN HUMAN SEMEN (PLASMA)

Document ID: FP09 I87 R01 B.5 Update: 1/07/2020

ABBREVIATIONS

CLSI	Clinical and Laboratory Standards Institute
CV	Coefficient of Variation
IVD	In Vitro Diagnostic Device
LOD	Limit Of Detection
LOQ	Limit Of Quantification
OD	Optical Density
PNP	Para (4)-Nitrophenol
PNPG	Para (4)-Nitrophenyl-alpha-D-glucopyranoside
SDS	Sodium dodecyl sulfate
WHO	World Health Organization

INTENDED USE

EpiScreen Plus™ is an In Vitro Diagnostic Device (IVD) for the quantitative measurement of neutral alpha-glucosidase in human semen (plasma). The enzymatic activity of at least 25 samples can be assessed with one EpiScreen Plus™ kit.
For professional use only.

GENERAL INFORMATION

The bulk of alpha-glucosidase activity in semen, and more particularly that of its neutral iso-enzyme, depends on secretion by the epididymis[1]. In patients with azoospermia and normal androgen levels in peripheral blood, neutral alpha-glucosidase activity in semen plasma is a reliable marker of the epididymal contribution to the ejaculate.

Azoospermic males with bilateral obstruction between the epididymis and the ejaculatory duct have very low alpha-glucosidase activity in their seminal plasma[2]. In contrast, if azoospermia is due to an arrest of sperm maturation, or obstruction situated between the epididymis and the rete testis, or in the rete testis itself, alpha-glucosidase activity is normal. Hence, neutral alpha-glucosidase assessment in seminal plasma of normally virilized men with azoospermia can differentiate between the major causes of this condition[3, 4].

Low neutral alpha-glucosidase activity in seminal plasma of patients with oligozoospermia may reflect partial obstruction of the epididymis associated with infections or inflammatory disease[2, 5]. Enzyme activity in patients with normal sperm concentration is correlated with the result of the Shorr-stain of mid-piece and tail, reflecting changes in the sperm membrane, induced by epididymal secretion [5].

The EpiScreen Plus™ assay may assist in the diagnosis and the management of male infertility.

ASSAY PRINCIPLE

The principle of the test is based on the following reaction:

PNPG + alpha-glucosidase → alpha-D-glucopyranoside + PNP (yellow)

Under specified conditions (pH=6.8; T=37°C), 1 IU of alpha-glucosidase liberates 1 µmol PNP per minute from substrate PNPG[7]. The yellow colour of PNP can be measured spectrophotometrically at 405 nm. Alpha-glucosidase activity is expressed as IU/Liter (or mIU/mL).

The reaction buffer contains SDS, which selectively inhibits the acid form of alpha-glucosidase originating from the prostate. This allows specific determination of neutral enzyme activity[6].

Inhibition: Glucose inhibits alpha-glucosidase by binding to the monosaccharide binding site of alpha-glucosidase[8]. This inhibition process is a pH and dose-dependent phenomenon, and is the principle behind creating control semen (plasma) samples.

SPECIMEN TYPES

The assay can be performed on fresh or frozen/thawed semen and seminal plasma samples.

MATERIAL INCLUDED IN THE KIT

- Reagent 1 (5ml): reaction buffer (pH 6.8), supplemented with 1% SDS
- Reagent 2 (0.25ml): 50x substrate solution (PNPG in DMSO)
- Reagent 3 (5ml): inhibitor solution (reaction buffer containing glucose)
- Reagent 4 (60ml): stopping buffer (0.02M NaOH)
- Reagent 5 (1ml): standard stock solution (5mM PNP)
- Reagent 6 (60ml): standard dilution buffer (0.02M NaOH + 0.1% SDS)

A certificate of analysis and MSDS are available on request or can be downloaded from our website (www.fertipro.com).

MATERIAL NOT INCLUDED IN THE KIT

Plate reader, photometer (405nm filter), thermoshaker or warm water bath, pipettor,1.5ml Eppendorf tubes, microtitre plate

STORAGE, TRANSPORTATION AND STABILITY

Suitable for transport or short term storage at elevated (up to 5 days at 37°C) temperatures. EpiScreen Plus™ must be stored at 2-8°C, protected from (sun)light, and remains stable for 24 months (even opened). Do not use after expiry date.

ASSAY PERFORMANCE

Validation parameters have been calculated based on the CLSI guidelines[9,10].

Measuring range: 2.32-144 mIU/ml	
Intra-assay CV: 3.08 %	Sensitivity: 96.0 %*
Inter-assay CV: 10.52%	Specificity: 93.6 % *
Cutoff: 6.35 mIU/ml;	
WHO lower reference limit: 20 mIU/ejaculate (if corrected for ejaculate volume)	

* vasectomized/normozoospermic

PRE-USE CHECKS

Do not use the product if seal of the container is opened or defect when the product is delivered. When stored between 2-8°C, precipitation may occur in Reagent 1 but disappears by pre-warming to 37°C.

METHOD

We recommend to watch our demonstration video (download via link on our website, or scan barcode):

Note 1: The WHO advises to apply only two internal quality control samples for blank correction. Because background variance of semen samples is quite large (+/- 20%), we recommend preparing a negative control for each semen (plasma) sample to allow correct and reproducible background correction.

Note 2: when reagents or samples need to be warmed or incubated, always use a thermo-regulated warm water bath or a fitting reaction tube thermoshaker or heatblock. DO NOT incubate in an air incubator as this may impair assay outcome.

Perform the following steps:

1. Warm reagents 1, 2 and 3 up to 37°C for 30 minutes (warm water bath, thermoshaker or heatblock)
2. For each semen (plasma) sample to be analyzed:
   - make reaction solution: 3µl of substrate solution (Reagent 2) in 147µl of reaction buffer (Reagent 1).
   - make inhibitor solution: 3µl of substrate solution (Reagent 2) in 147µl of inhibitor solution (Reagent 3).
3. Pipette 20µl of each semen (plasma) sample into two 1.5ml Eppendorf tubes.
4. Add 130µl reaction solution to one reaction vessel and 130µl inhibitor solution to the other (for negative control).
5. Vortex and incubate for exactly 2h at 37°C in a warm water bath or heatblock.
6. During incubation of the semen (plasma) samples, prepare the dilutions for the PNP-standard curve:
   a. Make the highest standard of 200 µM: dissolve 100 µl of standard stock solution (Reagent 5) in 2400µl of standard dilution buffer (Reagent 6). Mix gently.
   b. Use this solution to prepare the other standards, as indicated in the table below. Reagent 6 alone serves as 0 standard (blank).

Standard dilutions of PNP

PNP standards	200 µM Standard	Reagent 6
200 µM	**500 µl**	**0 µl**
150 µM	**375 µl**	**125 µl**
100 µM	**250 µl**	**250 µl**
50 µM	125 µl	375 µl
10 µM	25 µl	475 µl
0 µM (= blank)	0 µl	500 µl

7. After 2h incubation of the samples (reaction and inhibitor), stop the reaction by removing the tubes from the heatblock/warm water bath, adding 1ml of the stopping buffer (Reagent 4), and vortexing.

8. Pipette 200µl of all samples and standards (prepared in step 6) into a microtitre plate.
9. Read absorbance in a photometer at 405nm.

CALCULATION OF RESULTS

Download the Excel calculation sheet from our website and enter data in the sheet to calculate results:

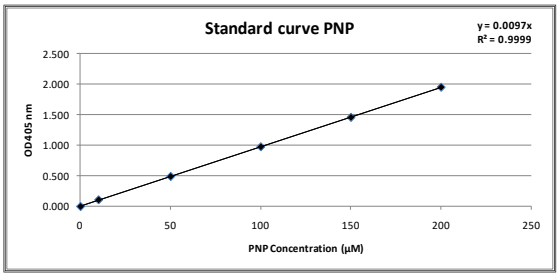
https://fertipro.com/2019/11/12/episcreen-plus/

PRINCIPLE:

1. Correct all measured OD values for the blank OD value (0 µM PNP standard). Only these corrected values will be further used in the next calculations.
2. Calculation of PNP-standard curve, with the standard concentrations in the X-axis and the corrected OD values in the Y-axis. Then, linear regression is performed to calculate the slope. Coefficient of determination (R^2) should be ≥ 0.99.
3. For each reaction sample: correct for its seminal plasma background (= Corrected $OD_{REACTION}$ – Corrected corresponding $OD_{INHIBITOR}$)
4. Use equation of the regression curve to calculate PNP concentration of the unknown sample
   (PNP concentration = background-corrected OD value / slope)
5. Calculate enzyme activity (in mIU/ml) by multiplying the PNP concentration with 0.479 (see section "correction factor" below)
   Calculated enzyme activity can be multiplied with ejaculate volume, to evaluate enzyme activity in the whole ejaculate.

Example

Assay data and standard curve:



Slope of the curve = 0.0097 (equation curve: y= 0.0097x), R^2 = 0.9999
Blank OD (0 µM PNP standard) = 0.045;
$OD_{REACTION}$ = 0.845 → corrected for the blank: 0.845 - 0.045 = 0.800
$OD_{INHIBITOR}$ = 0.060 → corrected for the blank: 0.060 – 0.045 = 0.015
$OD_{BACKGROUND\ CORRECTED\ SAMPLE}$ = 0.800 – 0.015 = 0.785
Concentration PNP = 0.785 / 0.0097 = 80.93 µM
Enzyme activity per ml = 80.93 µM x 0.479 = 38.76 mIU/ml
Enzyme activity per ejaculate = 38.76 mIU/ml x ejaculate volume (ml)

Note 1: The standard curve consists of points between 0-200 µM, as most semen samples will have values within this range. Linearity of the curve has been shown up to 300 µM however. If desired, the operator can alter the curve by starting at 300 µM, corresponding to an enzyme activity of 144 mIU/ml.

Note 2: the correction factor of 0.479 has been established based on the sample dilution factor and incubation time (120 min):

The assay uses 20µl of the semen sample, which is diluted in reagents to 1150µl, yielding a dilution factor of 57.5. One enzyme unit is defined as the formation of 1 µmol PNP per minute. Therefore, the dilution factor is divided by 120 to calculate activity per minute. This results in a final factor of 0.479.

WARNINGS AND PRECAUTIONS

This test is an aid in the diagnosis and, as for other biological tests; interpretation of the results must be performed within the framework of clinical findings and data of history taking. Other causes of insufficient epididymal secretion must be excluded, such as hypo-androgenism or severe testicular atrophy.

All materials must be handled in a safe way according to local/national norms.

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 reagents (gloves, lab coat, eye/face protection).

BIBLIOGRAPHY

1. Cooper TG, Yeung CH, Nashan D, Jöckenhovel F, and Nieschlag E. (1990) Improvement in the assessment of human epididymal function by the use of inhibitors in the assay of alpha-glucosidase in seminal plasma. *Int. J. Androl.*, 13: 297-305

2. Guerin JF, Ben Ali H, Rollet J, Souchier C, and Czyba JC. (1986) Alpha-glucosidase as a specific epididymal enzyme marker. Its validity for the etiologic diagnosis of azoospermia. *J. Androl.*, 7: 156-162

3. Casano R, Orlando C, Caldini AL, Barni T, Natali A, and Serio M. (1987) Simultaneous measurement of seminal L-carnitine, alpha 1-4-glucosidase and glycerylphosphorylcholine in azoospermic and oligospermic patients. *Fertil. Steril.,* 47: 324-328

4. Cooper TG, Yeung CH, Nashan D, and Nieschlag E. (1988) Epididymal markers in human infertility. *J. Androl.*, 9: 91-101

5. Haidl G, Badura B, Hinsch KD, Ghyczy M, Gareiss J, Schill WB. (1993) Disturbances of sperm flagelle due to failure of epididymal maturation and their possible relationship to phospholipids. *Hum. Reprod.*, 7: 1070-1073

6. Paquin R, Chapdelaine P, Dubé JY, Tremblay RR (1984) Similar biochemical properties of human seminal plasma and epididymal alpha-1,4-glucosidase. *J. Androl.*, 5: 227-282

7. WHO laboratory manual for the examination and processing of human semen, 10th edition. Measurement of neutral alpha-glucosidase in seminal plasma. pp. 134-136

8. Yao X, Mauldin R, Byers L. (2003) Multiple sugar binding sites in α-glucosidase. Biochim. Biophys. Acta, 1645: 22-29

9. Shrivastava A, Vipin B, Gupta VB (2011) Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chron. Young Sci. 2: 21-25

10. Chesher D. (2008) Evaluating Assay Precision. Clin Biochem. Rev., 29: S23-S

11. Eertmans F, Bogaert V, Van Poecke T, and Puype B. (2014) An Improved Neutral a-Glucosidase Assay for Assessment of Epididymal Function - Validation and Comparison to the WHO Method. Diagnostics. 4: 1-11

TECHNICAL SUPPORT

FertiPro NV
Industriepark Noord 32
8730 Beernem
Belgium

CE IVD REF EPI_PLUS