

INSTRUCTION & USER MANUAL



Test Package Content

1. Estrogen Biotransformation
2. Test Procedure
3. Patient Information



**BIOTRANSFORMATION AND
OXIDATIVE STRESS STATUS PROFILE**

The top right of the page features a decorative graphic composed of several overlapping light green hexagons. Some hexagons contain grayscale images: one shows a close-up of various pills, another shows a doctor in a white coat with a stethoscope around their neck, and a third shows a person's hands. The hexagons are arranged in a cluster, with some partially cut off by the edge of the page. There are also a few smaller, solid green hexagons scattered around the main cluster.

BACKGROUND INFORMATION

Estrogen is the primary female sex hormone that is responsible for the development and the regulation of the reproductive system in females. These hormones are also present in males in lower concentrations. Although estrogen is a naturally occurring hormone, synthetic estrogen products are also available and used in combined oral contraceptives and hormone replacement therapy. After having done its work, both natural estrogen and synthetic estrogens are metabolized to form breakdown products that can easily be excreted from the body.

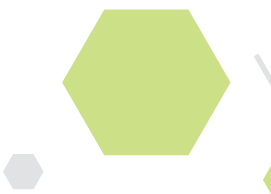
This breakdown process is called biotransformation and may follow different routes. The one route yields 2-methoxyestradiol, which has been shown to have anti-cancer properties, while the other routes yields metabolites that may damage the DNA and increase the risk to develop cancer. Disturbances in the biotransformation of estrogen due to hormone overload or genetic variations that influence the efficiency of the process may lead to an increase in the production of the toxic DNA-damaging estrogen metabolites.



NORMAL LIVER BIOTRANSFORMATION

Various other substances that are either in excess or toxic to our bodies, also undergo biotransformation. In today's world of processed food and pollution, toxic substances exist almost everywhere. They are in the food we eat, the water we drink, and the air we breathe. It is estimated that 60 to 80 percent of all cancers are a direct result of these environmental toxins. Environmental toxins may also play a role in the development of neurological disorders such as Alzheimer's and Parkinson's disease, and mental or behavioral disorders such as depression, anxiety, schizophrenia, and attention deficit disorder.

The liver is a key organ in your body's self-defense system. Inside the liver cells, there are sophisticated mechanisms in place to break down excess or toxic substances into forms which your body can safely eliminate. The liver uses two mechanisms called phase I and phase II biotransformation, designed to convert fat-soluble toxins into water-soluble substances so that your body can easily excrete these substances via watery fluids such as bile and urine.



In phase I, your body's biotransformation enzymes convert a substance into a product which can be further metabolized in phase II. This is achieved by various chemical reactions and during this process, antioxidants (such as vitamin C and E) can reduce the damage caused by free radicals. If antioxidants are low and toxin exposure high, these toxins become more dangerous because they cannot be eliminated efficiently, and then **oxidative stress** may arise. Slower biotransformation results in more toxic substances circulating in the body which are eventually stored for years in fatty body tissue, and can be released during times of exercise, stress or fasting. Phase II is also called the conjugation pathway whereby other enzymes in liver cells add another substance to metabolites from phase I. This makes the toxin water-soluble and less harmful, so it can then be excreted from the body via watery fluids such as bile or urine.



ESTROGEN BIOTRANSFORMATION

Estrogen also undergoes phase I and phase II biotransformation. The enzymes involved in these reactions are not only present in the liver cells, but also in other tissues, e.g. breasts, ovaries, adrenal glands, and uterus. In the biotransformation of estrogens, estrogenically active molecules can be bound to sulfate or glucuronides and be excreted. Estrogens can also form 2-, 4- or 16- hydroxylated estrogens. The hydroxyl estrogens can be converted to very reactive and dangerous molecules that can react with DNA and lead to DNA adduct formation. Instead of that route, 2- and 4-Hydroxyestrogens can be bound to methyl groups to prevent their conversion to more toxic metabolites. Conjugation of the very reactive molecules with the antioxidant glutathione, can also protect against DNA adduct formation. Glutathione conjugates are then catabolised to be excreted.

At the Centre for Human Metabolomics we can measure the different breakdown products of biological estrogens that are excreted in the urine. Therefore, by determining the relative levels of these metabolites, we can tell when someone produces more of the DNA-damaging estrogen metabolites.



THE TEST OUTCOME

The results that we obtain from analyzing the urine sample swab, give us the levels of urine:

- All 3 estrogens (Estrone, Estradiol, Estriol)
- Sulfate and glucuronide estrogens
- Hydroxyl estrogens
- Methoxy estrogens
- Estrogen DNA adducts
- Additional results:
 - Urine levels of 3 other non-estrogen hormones (Progesterone, Testosterone, Androstenedione)
 - Urine creatinine values
 - Glutathione conjugation estimation



The results will allow interpretation on:

- Can the liver effectively detoxify the load of estrogens?
- What route is primarily followed in the breakdown of estrogen?
- If dangerous routes lead to DNA adduct formation.



ADDITIONAL RECOMMENDATIONS:

Biotransformation and oxidative stress status test

The normal liver Biotransformation and oxidative status (BOSS) test, is also recommended in conjunction with the estrogen metabolite analysis. With the BOSS test, we can assess the liver's overall biotransformation function as well. During this test, the liver is challenged by common substances such as caffeine, aspirin and paracetamol in safe doses and samples of blood, urine and saliva are collected. This test is unique because phase I and II biotransformation pathways, as well as the oxidative stress status (which is affected by free radicals) and antioxidant capacity, are assessed. Phase I activity is assessed on saliva samples using the caffeine clearance test, and for phase II the production of four conjugates are determined in the urine. The ratio between the activities of the two phases is also calculated to detect imbalances. Finally, blood samples are used to determine the oxidative stress status and antioxidant capacity. Feel free to speak to your doctor or contact Green Health should you wish to request the BOSS test as well.

2. TEST PROCEDURE



It is especially important that the test procedures are strictly followed.

The sample (sample swab in sample holder), questionnaire, drying rack and tweezers included in the kit should be delivered to Green Health Potchefstroom. The delivery can either be done personally or by using the courier bag included in the kit (free of charge). From Green Health samples will be collected for analysis by the Biotransformation and oxidative stress status laboratory, (Human metabolomics) at the North-West University, Potchefstroom Campus.





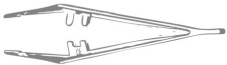
ABOUT THE TEST KIT AND SAMPLING CONDITIONS

For the urine sample collection, the following conditions apply:

- If premenopausal and not using any hormones, urine should be collected during the luteal phase of the menstrual cycle between day 18 and day 24. See the instructions below (Pre-test instructions) on how to calculate cycle day.
- If postmenopausal or using any type of hormonal supplements, this should be clearly indicated on the form, and urine can be collected on any day possible.
- Preferably, first-morning urine should be collected.
- Urine sample collection should not be done during a urinary tract infection

Adherence to these conditions are important to compare measurements to our reference range to ensure that fluctuating metabolite levels are within the detectable range. However, if urine samples were not taken as described above, this is not a problem: samples can still be analyzed, and results interpreted.

The provided kit consists of the following:

ITEM	STEP IN WHICH USED	DESCRIPTION
<p><i>Information booklet</i></p> 	<p>All steps to be followed are indicated in the booklet</p>	<p>Read the instructions in the information booklet carefully before starting the test. You can also keep this booklet for yourself or to recommend it to another patient.</p>
<p><i>Urine container with 1 sample swab</i></p> 	<p>Steps 1 - 3</p>	<p>Do not remove the sample swab from the container before step 3 listed below. Try not to touch the sampling swab with your hands. The urine container can be discarded after step 3.</p>
<p><i>Tweezers</i></p> 	<p>Steps 3 – 5 & 8</p>	<p>The tweezers should be used to transfer the swab from the urine container. You can rinse them between steps with tap water. The tweezers should be shipped back to the laboratory together with the sample after use.</p>

Drying rack



Step 3-4
& 8

The drying rack included is for drying the sample swab before shipping. This rack should be shipped back to the laboratory together with the sample after use.

Sample holder with moisture absorber pallets

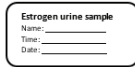


Steps 4-5
& 8

The sample holder should be used to ship the dried sample swab. Please ensure this sample holder is properly closed before sending.

Please do not remove the moisture-absorbing pallets from this holder.

Label



Estrogen urine sample
Name: _____
Time: _____
Date: _____

Step 6

Please complete all the details on the label using a ballpoint pen (to avoid smudging).

Questionnaire



Step 7

Patient information questionnaire (3) should be fully completed and shipped with the samples provided. Samples received with incomplete questionnaires will not be analysed.

Courier bag



Step 8

Courier bag will be used to send the sample box containing the sample holder with the sample, tweezers, drying rack and questionnaire back to us free of charge

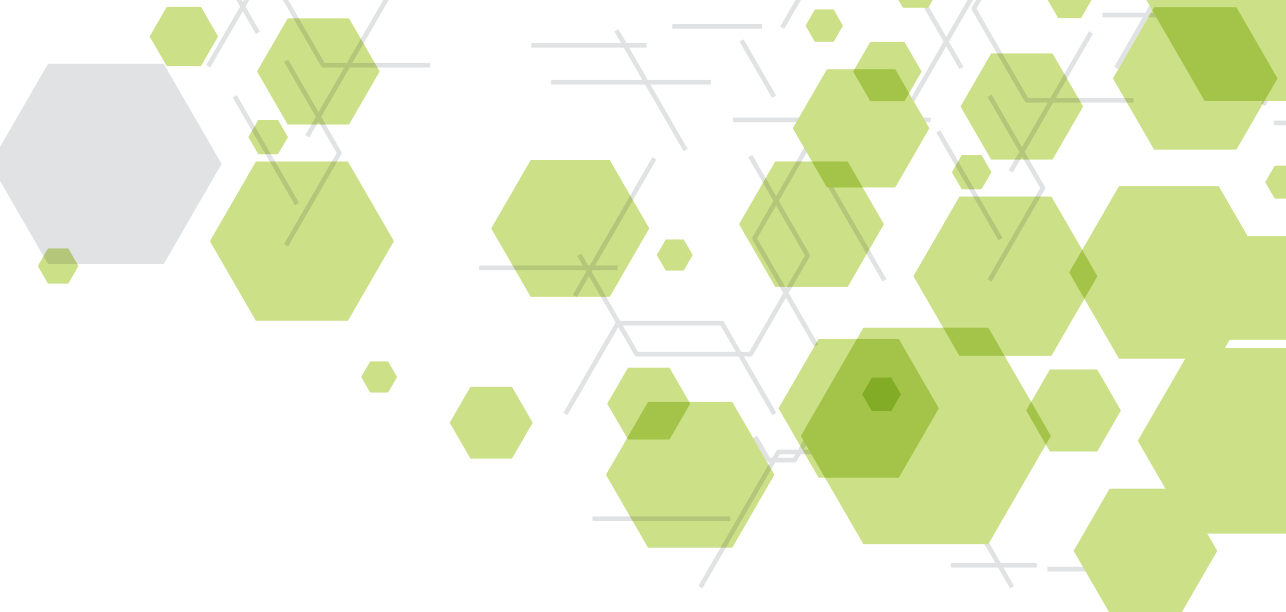


PRE-TEST INSTRUCTIONS

During this test, a normal diet can be assumed.

Calculate your menstrual cycle day:

Day 1 of your menstrual cycle is your first day of menstrual bleeding. For an average cycle length of 28 days, ovulation occurs around day 14. The period between ovulation and the next bleeding is the luteal phase (day 15-28). Between day 18-24, the estrogen levels remain relatively stable. Therefore, samples should be taken during this time, preferably on day 20 of the cycle. Day 20 will, therefore, be 19 days after your menstrual bleeding has started. If your average cycle length is not 28 days, samples should be collected as follows:



<i>Cycle length</i>	<i>Ovulation period</i>	<i>Sample collection day</i>
22 days	Day 6-10	Day 14-15
24 days	Day 8-12	Day 16-17
26 days	Day 10-14	Day 18-19
28 days	Day 12-16	Day 20-21
30 days	Day 14-18	Day 22-23
36 days	Day 20-24	Day 28-29
42 days	Day 26-30	Day 34-35

INSTRUCTIONS ON SAMPLE COLLECTION:

STEP 1



Collect in the urine container provided, without taking the sampling swab out of the tube. First morning urine is preferred.

STEP 2



Close the urine container and let stand for 10 min.

STEP 3



Using the tweezers provided, carefully take out the sampling swab and place it on the drying rack and leave the sample swab to dry. Avoid the sample swab touching anything else but the tweezers and the drying rack.

STEP 4



The urine swab must be allowed to dry completely after collection.

Air-Drying the swab for at least 24-36 hours in the well-ventilated area as left in step 3. (Very humid conditions may prolong this drying time)

STEP 5



After drying the sample, use the tweezers to transfer the entire sample swab into the top part of the sample holder with moisture absorber pallets provided. The top cap can be removed by pulling it away from the sample holder and closed by pressing it back into the sample holder.

Do not under any circumstance remove the second/bottom cap.

STEP 6

Estrogen urine sample
Name: <u>Jane Doe</u>
Time: <u>0800</u>
Date: <u>10/01/2019</u>

Complete sample label and apply to the sample holder containing the dry sample swab from step 5.

STEP 7



Complete patient information questionnaire (3) provided.
The information booklet can be kept, but the questionnaire should be sent back with your sample



Courier the following items in the sampling kit box back to us:

- Labelled sample holder with the sample
- Tweezers
- Drying rack
- Questionnaire

STEP 8



Place the kit box with all the items to be sent back to us in the courier bag provided and hand it in at any Aramex or Pick n Pay in your area.



Shipping address:

Monisha Green Health
72 Steve Biko St,
Noordburg,
Potchefstroom,
2522
018 297 1019

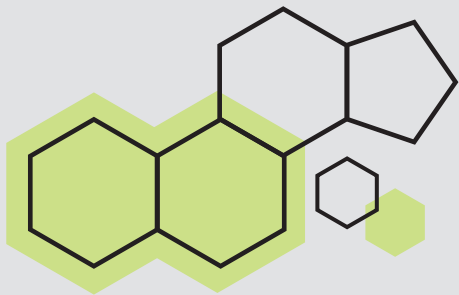


AFTERWARDS



Throw all remaining urine down the toilet. Close the empty urine container and discard in trash. Thoroughly clean the working surface used. Wash your hands.





CONTACT INFORMATION

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