



TECHNICAL FILE

viola Super Early Pregnancy Test from day 8

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1 Introduction

Human chorionic gonadotropin is a glycopeptide hormone produced by the placenta after conception and appears in the pregnant woman's blood or urine. Its rapid rise in the concentration during pregnancy makes it a good marker for confirming pregnancy. Following conception, the concentration of hCG in urine increases steadily and reaches a circulation peak of about 230 000 International units per litre between the 8th and 11th week of pregnancy. After delivery, these levels fall back to normal within a couple of weeks.

The *viola Super Early Pregnancy Test from day 8* has a sensitivity of 5 mIU/ml and is thus twice as sensitive compared to established early pregnancy tests.

1.1 Intended use

The *viola Super Early Pregnancy Test from day 8* is a rapid immunochromatographic assay for the detection of pregnancy at the very earliest stage by determining hCG in urine samples and can be used even before the absence of a menstrual period, from 8 days after conception.

A considerable number of pregnancies fail during the first weeks. However, learning that pregnancy has been achieved from the very earliest stage can prevent delays in prenatal care.

2 Description of the device

2.1 Housing

viola test devices consist of a two-piece moulded plastic housing, containing a test strip as the functional component.

The materials used for the sticks are dyed styrene-acrylonitrile copolymers.

2.2 Test strips

The test strips (dimensions: ~ 90 x 5 mm) consist of 5 overlapping layers: backing, sample pad, conjugate pad, nitrocellulose pad and wick. Coloured top tapes are used to protect the test strip.

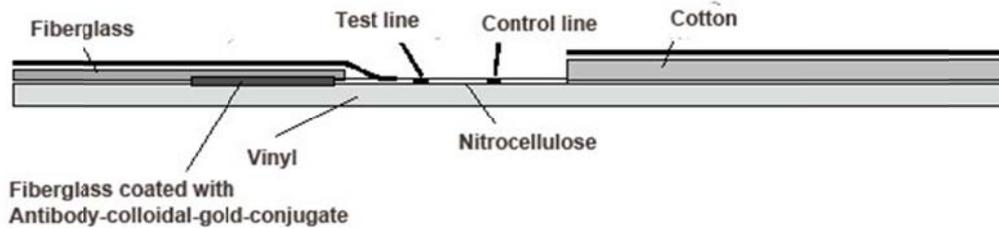


Illustration 1: Test strip scheme

2.2.1 Test principle

The test strip incorporates a monoclonal antibody-colloidal gold conjugate specific to β -hCG to bind to hCG on the one hand, and, on the other hand to a surface coated with monoclonal antibodies specific to α -hCG to capture the complex. As the urine sample is applied onto the test pad of the strip, the antibody coated surface captures intact hCG bound to the antibody-colloidal gold conjugate, forming a complex and giving a purple test line as soon as the urine sample contains **5 mIU hCG/ml**. In the absence of intact hCG in the sample, independent unbound conjugate binds to an antibody in the control area of the nitrocellulose pad and appears as purple line, confirming the correct test performance.

2.2.2 Active ingredients

Bovine Serum Albumin

Murine Anti- β -hCG monoclonal antibody-colloidal gold conjugate

Rabbit IgG monoclonal antibody-colloidal gold conjugate

Murine Anti- α -hCG monoclonal antibody

Goat Anti-(Rabbit IgG) polyclonal antibody

3 Test performance and interpretation

Test sticks are either dipped in a urine sample or held under a flow of urine for about 3 seconds. Results are evaluated after 10 - 15 minutes.

It is recommended that an early-morning urine sample be used.

If only one purple line, the control line, appears in the results field, this indicates that the specimen does not contain detectable levels of hCG and should therefore be considered as negative result.

If two purple lines appear in the results field, this indicates that the specimen contains hCG in sufficient quantities. This provides an indication of pregnancy, especially if the test line is only very faint. Tests performed before the absence of the menstrual period and which result in only a very faint test line should be repeated after a few days in order to confirm pregnancy.

If there is no line after 15 minutes, if there is only a test line or if the whole results field turns purple the test is considered invalid. *Invalid results mostly occur due to improper test performances.*

4 Limitations

In case of highly diluted urine, the test may show a negative result.

A considerable number of pregnancies result in miscarriages particularly within the first four weeks. This may lead to seemingly false-positive results.

The test cannot distinguish between normal and ectopic pregnancies. In both cases, a positive test result may occur.

Infertility treatment always involves hCG injections. This could lead to increased hCG values in urine and thus affect the test result.

Increased hCG levels are also observed in patients suffering from trophoblastic diseases, carcinomas, some non-trophoblastic neoplasms and bladder moles. In such cases the viola test might show a false positive result.

5 Contents of the test kit

viola Super Early Pregnancy Test from day 8

Box with foil pouch containing 1 test stick and 1 desiccant, instruction for use

viola Super Early Pregnancy Test from day 8, double pack

Box with 2 foil pouches, each containing 1 test stick and 1 desiccant, instruction for use

5.1 Dimensions of packaging materials

Foil pouch 17,8 x 6,2 cm / 16,8 x 7 cm
7 x 2.44" / 6.61 x 2.76"

Box 6 x 15 x 2,5 cm
2.36 x 0.98"

5.2 Symbols used for labelling

Foil pouch



Box



^{*)} incl. number of according notified body

6 Study data

6.1 Performance data

Sensitivity

The *viola Super Early Pregnancy Test from day 8* provides an indication of pregnancy as soon as the hCG level in urine reaches 5 IU hCG/l and above.

For this study, hCG standards calibrated against the WHO 3rd IS 75/537 were used as hCG positive controls in order to confirm the sensitivity level of 5 IU/l. Studies were performed for the respective product on 3 batches. The results were read visually after 10 minutes for each batch as follows:

hCG sample [mIU/ml]	Result expected	Result read
0	negative	negative
5	weak positive	weak positive
12,5	weak positive	weak positive
25	positive	positive
100	positive	positive
450000	positive	positive
900000	positive	positive
1500000	negative – weak positive *)	weak positive *)

*) 1500000 mIU/ml are not encountered in the physiological range. If very high concentrations of an analyte are applied, the immunoassay will show a false negative result (prozone effect).

Specificity

A cross reactivity study was performed in order to confirm the specificity of viola pregnancy tests.

hLH, a glycoprotein which triggers ovulation at midcycle, was added to hCG-free urine samples at the levels 50, 100, 300 and 500 mIU/ml (WHO 2nd IRP/hMG) and applied to viola pregnancy tests according to the instructions. All tests showed a negative result after 10 minutes. No cross-reactivity was observed at these concentrations.

FSH, a glycoprotein which is found at elevated levels in post-menopausal women was tested as well at 50, 100, 500 and 1000 mIU/ml (WHO 2nd IRP/hMG) hCG-free urine specimens. viola pregnancy tests showed consistently negative results at all levels. No cross-reactivity was observed with FSH.

TSH was tested in hCG-free urine samples at levels of 100, 500 and 1000 mIU/ml (WHO 63/38), consistently giving negative results.

The same concentrations of hLH, FSH and TSH tested with hCG-free samples were added to urine samples containing hCG at concentrations of 12,5 and 450 000 mIU/ml. A weak positive result was obtained with the sample containing 12,5 IU hCG/ml, positive results were yielded with the samples containing 450 000 IU/ml. Interferences through hLH, FSH and TSH are excluded.

Further, the following substances were tested with hCG-free as well as with hCG-added urine samples (0, 12,5 and 450 000 IU hCG/ml):

Acetaminophen, 20 mg/dl
Ascorbic acid, 20 mg/dl
Acetylsalicylic acid, 20 mg/dl
Atropine, 20 mg/dl
Caffeine, 20 mg/dl
Gentisic acid, 20 mg/dl
Ampicillin, 20 mg/dl
Tetracycline, 20 mg/dl
Albumin, 2000 mg/dl
Glucose, 2000 mg/dl
Human serum protein, 2000 mg/dl
Uric acid, 10 mg/dl
Cortisol, 200 ng/ml
Estradiol (E2), 25 ng/ml
Estriol (E3), 25 ng/ml
DHEAS, 500 ng/ml

In no case interference with the expected pregnancy test result was observed.

6.3 Laymen studies

Studies were performed in order to prove the ability of laymen to properly perform the viola Pregnancy Test and to correctly interpret a result in compliance with the instructions and without the assistance of a supervisory person.

Samples used were negative and hCG-weak positive solutions. Each participant could choose whether to perform the test directly or with a cup (the direct method was performed with the assistance of a dispenser).

A) Of 64 tests performed, there were 2 invalid test results due to not observing the maximum fill level (tests were flooded).

One of the 64 participants read out a negative result before the incubation period had expired. After 7 minutes the test showed a positive result though. As given in the instructions, a negative result cannot be determined before the end of the reaction time.

B) Of 61 tests performed, there were 3 invalid results: 2 test strips were flooded due to not observing the maximum fill level and 1 test stick was held at the lower end, this is why the sample could not get inside the housing. One test result was evaluated as negative despite a faint line was observed by the supervisory person.

It can thus be concluded that the instructions are comprehensibly formulated and understandable for laymen of all ages (16 – 61).

7 Stability and Storage

Real time stability studies were performed for each product on 3 batches. Tests were stored at room temperature (15 – 30 °C), sealed in foil pouches with a desiccant, and tested each month over a period of 2 years.

After a period of 24 months all tests met the specifications. viola pregnancy test devices (detection limit 12,5 IU hCG/l) are stable for a minimum of 24 months if stored at room temperature (s. table on page 9).

Month	LOT #1			LOT #2			LOT #3					
	negative control	12,5 IU hCG/l	50 IU hCG/l	negative control	12,5 IU hCG/l	50 IU hCG/l	negative control	12,5 IU hCG/l	50 IU hCG/l			
Replicate		1	2		1	2		1	2			
1	-	+	+	++	-	+	+	++	-	+	+	++
2	-	+	+	++	-	+	+	++	-	+	+	++
3	-	+	+	++	-	+	+	++	-	+	+	++
4	-	+	+	++	-	+	+	++	-	+	+	++
5	-	+	+	++	-	+	+	++	-	+	+	++
6	-	+	+	++	-	+	+	++	-	+	+	++
7	-	+	+	++	-	+	+	++	-	+	+	++
8	-	+	+	++	-	+	+	++	-	+	+	++
9	-	+	+	++	-	+	+	++	-	+	+	++
10	-	+	+	++	-	+	+	++	-	+	+	++
11	-	+	+	++	-	+	+	++	-	+	+	++
12	-	+	+	++	-	+	+	++	-	+	+	++
13	-	+	+	++	-	+	+	++	-	+	+	++
14	-	+	+	++	-	+	+	++	-	+	+	++
15	-	+	+	++	-	+	+	++	-	+	+	++
16	-	+	+	++	-	+	+	++	-	+	+	++
17	-	+	+	++	-	+	+	++	-	+	+	++
18	-	+	+	++	-	+	+	++	-	+	+	++
19	-	+	+	++	-	+	+	++	-	+	+	++
20	-	+	+	++	-	+	+	++	-	+	+	++
21	-	+	+	++	-	+	+	++	-	+	+	++
22	-	+	+	++	-	+	+	++	-	+	+	++
23	-	+	+	++	-	+	+	++	-	+	+	++
24	-	+	+	++	-	+	+	++	-	+	+	++

- ... negative result, + ... weak positive result, ++ ... positive result

8 References

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