The Kit for Sperm count Detection (Colorimetric method)

Operating Instructions

INTRODUCTION

The kit is used for qualitatively detecting sperm count of human semen in vitro, and conducting auxiliary clinical diagnosis of the infertility and self-evaluation male fertility of pre-pregnancy.

SUMMARY

The kit for sperm count detection (Colorimetric method) used the inert glass fiber membrane with high water absorption and the pore size of less than 0.5um to filter the semen, and sperm cells will be trapped on surface of the first layer of the membrane, and we use the staining fluid that can quickly dye sperm cells, the darker of the color, the more of sperm count on the filter membrane, and the count of sperm in the semen will be higher. Through comparing with the reference color (20million/ml specified by WHO) of well B on the test board, we know that if color of the test well A is lighter than color of the color card, the sperm count is positive, and if color of the test well A is darker than the color card, the sperm count is negative.

SPECIMEN COLLECTION

1. Before testing, the subject shall mortify for 3-7 days; too short or too long abstinence time will affect the accuracy of diagnosis;

2. Using masturbation to expel the semen directly into a sperm collecting cup, and adopting water bath of 37 ℃ to liquefy for 15 minutes (the semen collecting room in hospital) or using a condom to collect semen during the sex life (family environment), to pour all the semen into the semen collecting cup, and liquefy it for 5-15 minutes at room temperature, that is, the semen is changed into fluid liquid from glue frozen state, which shows that the liquidation is completed;

3. After collection of the semen, semen shall be carried out a test within 2 hours. And if it needs to be tested again later, the specimen of semen shall be stored in a refrigerator at 2 to 8 ℃, but the storage time shall not be more than 12 hours. If the specimen needs to be stored for a long time, please store it in a refrigerator, but it can only be dissolved and used for one time.

4. When the test is completed, we'd better read the results within 5 minutes. Over time, the color will fade slowly, if we want to compare the results with test of the next time, we can take the

results with a camera to save them.

TEST PROCEDURE

Before testing, we must read the instructions completely first

1. Specimen collection of semen: Using masturbation to expel the semen directly into the sperm collecting cup, or using a condom to collect semen during the sex life, to pour all the semen into the semen collecting cup.

2. Liquefaction: To shake evenly the semen in the semen collecting cup, and standing it for 15 minutes, until the semen becomes fluid liquid.

3. Add the specimen: To take out an experimental board from a aluminum foil bag, and place it on the operation platform horizontally, using an accessory specimen-absorption pipe to absorb a tube of semen from the semen collecting cup, and dropping three drops of the semen into the white A hole, and then the semen saturates to surface of the membrane and dry.

4. Add blue staining fluid: To add one drop of blue staining solution to test well A , and let it soak for 1-2 minutes.

5. Add transparent washing buffer: Add two drops of transparent washing buffer to test well A, and let it soak for 1-2 minutes, and then read the results immediately.

6. Read the results: Read the color of test well A , compare colors of test well A to reference Well B, the darker of the color, the more of sperm count, and report the results.

INTERPRETATION OF RESULTS

Results of naked eye observation: Compare colors of test well A to reference Well B.

Negative reactive results: The color of test well A is darker than standard color of reference Well B, and which shows that count of sperm ≥20million/ml, and count of the sperm is normal.

Positive reaction results: The color of test well A is lighter than standard color of reference Well B, and which shows that count of the sperm≤20million /ml, such case belongs to oligospermatism (5million/ml≤ count of the sperm≤20million/ml); If well A is colorless, and which shows that count of the sperm≤5million/ml, such case belongs to severe oligozoospermia or azoospermia.

The subject whose test result is positive or can not be judged easlily shall be repeated the test in well C, the testing result of well A shall be the same with well C. If the results are different, and

the correction test shall be conducted in well D (to add 4 drops of semen into well D), and the results can be explained as the following table. The subject shall be repeatedly examined in 3-7 days after the first test, and consult male doctors about the obtained results.

Repeated test A C	Correction test D	Explanation
All are negative	Need not to do	Sperm count≥20million/ml
Positive and negative can not be defined	Negative	17million≤Sperm count≤21million/ml, to consult the doctors
Positive and negative can not be defined	Positive	Sperm count <20million/ml, to consult the doctors
All are positive	Need not to do	Sperm count <20million/ml, to consult the doctors

[Reference value (reference range)]

The laboratory test manual for interaction between WHO human semen and sperm- cervical mucus recommends using Neubauer blood cell counting plate as a tool for semen analysis. At present, the value is set in domestic and abroad: In normal semen, count of the sperm is more than 20 million/ml; the case that the count of sperm is less than 20million /ml belongs to oligozoospermia; the case between 5 million to 10 million/ml belongs to moderate oligozoospermia disease; and the case of less than 5 million/ml belongs to severe oligozoospermia.

PRECAUTION

1. This kit can only be used for diagnosis tests in vitro. To test human semen specimen and it does not apply to specimen of other body fluids.

2. The kit shall be stored at room temperature, to avoid moisture. If the foil packaging is damaged, please not use.

3. If the test card's package is opened, it shall be used as soon as possible, to avoid to be placed in the air for a long time, resulting in damp and failure.

4. The kit is a kind of product used for primary test, any measured positive results shall be determined with other methods.

5. When the tests are carried out for a large number of specimens, please make marks, to avoid confusion.

6. Adding specimens and cleaning shall try to avoid bubbles, resulting effects to the test results.

7. To observe the reaction results strictly according to time requirements of the instructions.

8. This kit can only be used for one-time diagnosis in vitro, and the semen taken from different subjects or from the same subject at different times can not be used repeatedly.

9. The kit must not be frozen or used after the expiry.

STORAGE AND STABILITY

The test kit can be stored at room temperature (18 to 30°C) in the sealed pouch to the date of expiration. The test kit should be kept away from direct sunlight, moisture and heat.

[The common questions and answers on the kit for sperm density detection]

If necessary, you may refer to screening test instructions of male infertility rapid diagnosis.

1. Why the abstinence time is 3-7 days before the test?

Answer: After ejaculation, sperm count in the epididymis needs to get back to normal for three days; if the abstinence time before the test exceeds 7 days, the matured sperm in the epididymis begins to apoptosis, and which may also affect the determination of sperm density.

2. Why the time is 15 minutes before the semen being taken out from the semen collecting cup for test, and the storage time after sampling does not exceeds 12 hours?

Answer: The fresh semen is viscous, and normal semen needs to be incubated for 30-60minutes at 37℃ to liquefy completely. About 30% of male patients have suffered from the symptoms of liquefaction bad with varying degrees, and the semen does not liquefy until the room temperature is up to 37 ℃, while only in liquid state, the semen can be used for test, because the viscous semen can not completely immerse into the test hole. The light yellow sheet at bottom of the semen collecting cup can make the semen liquefy quickly within 15 minutes. If the storage time of sperm specimen is too long, and which will cause the sperms lysis, to affect accuracy of the count.

3. How long time do the semen and reagent solution need to immerse the test hole?

Answer: In general, they may pass membrane of the test hole within several seconds, if they can not immerse the test hole completely after 5 minutes, and which shows that the semen has not

liquefied completely, or density of the sperm is too high, and we must repeat the test. The reasons of semen non-liquefaction may be that the enzyme in the semen collecting cup becomes invalid or the user does not use the sperm retrieval cup correctly.

4. Is the solutions used for test safe?

Answer: The solution is safe, and which is a synthetic dye proved that the intake amount with the concentration of less than 5mg/kg does not harm to human body. And concentration of the staining fluid is less than 10ug/ml.

5. Can the negative results show that the subject has ability to have children?

Answer: Not necessarily. The sperm count is one of the important indicators of reflecting the male fertility, but other factors except for sperm count can also cause male infertility. For the results of this test, you can consult the male doctors.

6. Can the positive results show that the subject has no ability to have children?

Answer: Not necessarily. If both of the two repeated tests are positive results, and which can show that the subject is an oligospermatism patient, whose natural fertility is significantly lower than normal male. The study has showed that the insemination success rate of the oligospermatism patient is less than half of normal male, therefore, the positive results can not show that the subject can not have children completely. If color of the results is very close to white, and which prompts that the user may be a azoospermia (no sperm)patient, and the azoospermia patient needs to go to hospital for further examination, to determine it is an obstructive azoospermia or azoospermia caused by dyszoospermia.

7. What is the reason that may result in wrong test results?

Answer: The subject does not mortify on time before the test, collection and storage of semen specimen are non-standard, the semen does not liquefy completely, the test has not been conducted as specified time of the operating steps or the sperm count is too high so that the seminal plasma can not be completely absorbed by the membrane, and all these are the reasons that can cause test failure or inaccurate diagnosis.