Instructions for Sperm Staining Solution (Diff-Quik Method)

[Product Name]

Sperm Staining Solution (Diff-Quik Method)

[Package Specification]

Specification 1: Fixation solution: 100 ml×1, Staining solution A: 100 ml×1, Staining solution B: 100 ml×1.

Specification 2: Fixation solution: 500 ml×1, Staining solution A: 500 ml×1, Staining solution B: 500 ml×1.

[Intended Use]

The solution is mainly used for sperm morphology staining analysis.

[Test Principle]

This solution takes advantage of the fact that the proteins with different isoelectric points in the cell can selectively bind the corresponding dyes and color at the same acidity with different charges.

[Main Component]

No.	Component name	Specification	Main ingredient
1	Fixation Solution	100 mL×1/500 mL×1	Contains triarylmethane dye
2	Staining Solution A	100 mL×1/500 mL×1	Contains Eosinophilic xanthene
3	Staining Solution B	100 mL×1/500 mL×1	Contains Basophilic thiazepineains

[Storage Conditions and Expiration Date]

Transported at room temperature, and can be stored room temperature(5℃~30℃) for 12 months; it is valid for 30 days after being opened and stored at 5℃~30℃.

[Applicable Instruments]

Suitable for Full Automatic Staining Machine (models RS-160, RS-161, RS-162) manufactured by Zhuhai Cariad Medical Technology Co., Ltd.

[Sample Requirements]

Freshly liquefied semen, or stored frozen at -20°C for test.

[Test Method]

1. Specimen preparation

1) Prepare the film immediately after complete liquefaction of the fresh specimen. If the liquefaction of the specimen is delayed or the viscosity is too high, a liquefying agent may be added to promote complete liquefaction, and the film may be produced after complete liquefaction.

2) Low-density, high-viscosity, debris-filled semen specimens require sperm washing followed by film preparation, and the method is as follows:

① Add 0.2~0.5mL of semen and 10mL of saline to a test tube.

② Centrifuge at 800g for 10min and remove the vast majority of the supernatant.

③ Gently flick the test tube to suspend the centrifuged sperm mass in the remaining saline, count the sperm density, and adjust the sperm density appropriately with saline and seminal plasma at a volume ratio of 1:2 (no more than 80×10^6/mL).

3) Semen taken from the refrigerator at 2-8°C should be equilibrated at room temperature for 30 min or 37°C for 5 min.

2. Operation method

2.1 Manual operation

1) Add a small drop of semen (5 to 20 μL) on a clean slide. If the sperm density exceeds 20×10^6/mL, 5 μL of semen should be taken; if the sperm density is less than 20×10^6/mL, 10~20 μL of semen should be taken.

2) When the consistency of semen is low, the "thinning" technique can be used to push the slide (using the edge of the second slide to push and pull a drop of semen forward on the surface of the clean slide); when the consistency of semen is high, drop a drop of semen in the center of one slide, and then cover the second slide with the surface down to spread the semen between the two slides, and gently pull to separate the two slides to prepare two slides at the same time.

3) Place the coated slides horizontally and dry them naturally in the air.

4) After the smear is dry, cover the slide with drops of fixation solution for fixation of 45 s. Place the slide upright on absorbent paper to remove excess liquid (do not rinse with running water).

5) Add drops of Staining Solution A to cover the slide and stain for 30 s. Place the slide upright on absorbent paper to remove excess liquid (do not rinse with running water).

6) Add drops of Staining Solution B to cover the slide, stain for 15 s, and rinse with purified water to remove excess dye.

7) Hold the slide upright to remove the water and allow it to dry completely.

8) Observe at least 200 sperms under a normal light microscope with the objective 100× oil lens and classify and count the morphology of sperms.

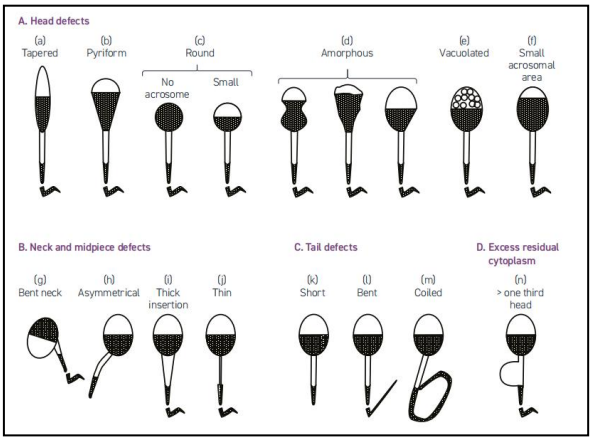
2.2 Staining machine operation

Select the quick staining program and perform the staining operation according to the operation manual of the Full Automatic Staining Machine.

[Result Calculation]

1. The acrosome region of the sperm will be stained light purple, the post-acrosome region will be stained dark purple, and the body and tail region of the sperm will be stained light red.

2. The World Health Organization (WHO) classifies the types of sperm defects as follows:



[Reference Value]

Normal sperm morphology ≥ 4%.

[Test Result Explanation]

The WHO recommends the following parameters for evaluating sperm morphological abnormalities: teratozoospermia index (TZI) or multiple anomalics index (MAI), that is, divide the number of deformed sperm by the total number of defects, and sperm deformity index (SDI), that is, the total number of defects divided by the total number of sperm. These parameters predict the function of sperm in vivo and in vitro.

[Method Limitations]

1. Only identifiable sperm with tails will be considered for counting of different morphological sperm; immature sperm cells including the round sperm cell stage cannot be counted as sperm. The sperms with detached heads or without heads will not be counted as sperm, but should be recorded separately.

2. This staining method may produce background color and requires centrifugation and washing; in addition, the head of sperm stained with this method is greater than that of Papanicolaou staining or Shorr staining.

3. Although the operation is simple, the morphological stability of the sperm after staining is not as good as that of the Papanicolaou staining.

[Product Performance Index]

1. Staining effect:

The acrosome region of the sperm is light purple, the post-acrosome region is dark purple, and the body and tail regions are light red.

Caution

1. Sometimes it is difficult to make a good smear, so if there is no abnormality in the viscosity of the seminal plasma, the "thinning" technique is often used for direct smearing, and then depending on the staining effect to decide whether the sperm need to be rewashed.

2. If the tissue slides fall off during the staining process, it may be due to the excessive thickness of the smear.

3. If the staining is light or uneven during the staining process, it may be due to the room temperature being less than 20°C or the semen not being equilibrated at room temperature for 30min or 37°C for 5min.

4. Since all samples from the subject should be considered as potential sources of infection, samples and reagents after testing must be disposed of as medical waste to avoid contamination of the environment.

5. The methanol and methylene blue contained in the kit are chemically hazardous and should be avoided by contact or inhalation.

6. The user of the product must have the professional qualifications required for inspection personnel.

7. The product should be used before the expiration date to ensure its effectiveness.

[Symbol Explanation]

Symbol	Symbol Note	Symbol	Symbol Note
	Manufacturer		In vitro diagnostic medical device
	Date of manufacture		Batch code
	Authorized representative in the European Community		Catalogue Number
	CE mark		Use-by Date
	Consult instructions for use		Temperature Limit
	Caution		Contains sufficient for <n> tests

[References]

World Health Organization (WHO), Laboratory Test Manual for Human Semen and Sperm-Cervical Mucus Interaction (Fourth Edition), People's Medical Publishing House, 2001.

[Essential Information]

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CE mark

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[Revision History]

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