Testsimplets® Prestained Slides
Application for Sperm Morphology

Intended Use

This innovative product eliminates the laborious and time consuming staining protocols currently in use. It is now possible to stain all the various cells present in a fresh semen specimen with little effort.

Summary and Explanation

The Testsimplets application allows a differential for a morphology evaluation of a semen specimen. It is based on the presence of two dyes, *new methylene blue N* and *cresyl violet acetate.* Present as chemically pure substances, the dyes are evenly applied to the glass slides in constant quantity and admixture. This ensures reliable and satisfactory structural staining differentiation. Traditional smearing techniques and dried preparations sometimes result in damage to cell structure.

Principle of procedure

The reaction of the two dyes with the seminal cells can be visualized as being analogous to panchromatic (panoptic) staining. The different staining of the individual cell regions with the basic dyes is used for cell differentiation.

Package Contents

The package contains:

- 50 Prestained ready-to-use slides (aluminum foil sealed packaging)
- 50 Dust-free coverglasses (24x36mm)(plastic sealed packing)

Storage and Expiration

Store package at ± 2° to 30° C.

When kept in the original pack, Testsimplets are stable up to the date specified on each pack. Once the pack has been opened, high atmospheric humidity and large fluctuations in temperature may lead to sporadic formation of crystals in the prestained layer. Although this does not affect the performance of the test, it is important to recluse the package immediately after having removed a slide.

Materials Needed but not Provided

- Pipette and tips or glass rod
- Microscope with l00x oil immersion objective
- 10% Formalin in coplin jar

Specimen Preparation

1. Allow the semen to liquefy at room temperature.
2. Immediately prior to application, mix the specimen thoroughly.

Slide Preparation

1. Take the two packs out:

- 50 **Slides:** Open the aluminum foil carefully by lifting the blue lid. The slides can now be removed by hand or with the aid of the dispenser. Do not touch the prestained area of the slide surface.
- 50 **Coverglasses:** Open the pack by slitting the label along the broken line and lift up the lid vertically in the direction of the arrow. Remove the coverglasses.

Procedure

1. Pipet 3-5µl of the well-mixed semen specimen directly to the center of the stained portion of the labeled slide.
   a. For spermatozoa density *less than 10 million/ml,* centrifuge the semen for 8 minutes at 250g. Place a 5µ drop of the sediment onto the slide.
   b. For spermatozoa density *greater than 100 million/ml,* use a smaller volume. Preparation of a thin smear is essential for effective evaluations. Viscous samples can result in incomplete immobilization of the spermatozoa and excessive movement of seminal fluid making interpretation under oil immersion difficult.
   c. Semen need not completely cover the stained area.

Note: if you are going to archive slides, see Steps 1-4 under Archiving Slides. Otherwise, proceed with Step 2.

Archiving Slides (Optional)

1. Air dry slide at this time
2. Fix with 10% formalin for one minute.
3. Coverslip optional for archiving (see Step 3 below)
4. Slides can be archived for up to 4 months.

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2. Place the provided coverglass on the specimen immediately.
3. Gently press center of the coverglass with a pencil or pen point to obtain a very thin uniform smear. Viscous samples can be encouraged to spread evenly by exerting mild pressure on the coverglass. Press the point of the pencil or pen on the center of the coverglass, drawing outward toward the corners.
4. The slide may be sealed with nail polish if desired.
5. It is ideal to read the prepared slide after 2 hours to complete immobilization of all the sperm cells and settle out the seminal fluid. Allow the prepared slide to stand at least 30 minutes at room temperature before evaluating.
6. Examine slides under oil immersion, using a l00x objective.
7. Count at least 100, preferably 200 spermatozoa. Classify them using WHO or Tygerberg 'Strict Criteria'. List and classify them according to abnormalities of the head, midpiece, and tail. Express the total number of each category as a percentage.
8. Note the presence of other cells. List and classify them as; immature germinal cells, white blood cells, or epithelial cells.

Normal Values of Results

World Health Organization: 30% or more with normal forms
Tygerberg 'Strict Criteria': 14% or more with normal forms

References

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