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### **Antisperm-Antibody (IgG)**

MAR Test  
(mixed antiglobulin reaction)

**Blue Colored Coated Latex Particles**

 SP/SFT/ASA-007-A



**User Manual**

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Turnaround time for test

- IgG Direct: 15min
- IgG Indirect: 90min



Store at: 2°C - 8°C after receiving

## Concept

**Spermatozoa** are **antigenic**. The **testis** is an **immunologically privileged site** (i.e. transplanted foreign tissue can survive for a period of time without immunological rejection) protected from access by either lymphocytes or macrophage.

However, **damage** to the **genital tract** may allow sperm to come in contact with immunologically competent cells, such as lymphocytes, & thus allow the **generation of antibodies** against **sperm surface antigens**.

Sperm **antibodies** may **interfere** with **spermatogenesis & sperm maturation** in the male, & hinder **sperm transport, cervical mucus penetration, capacitation, & fertilization** in the female.

Antisperm - antibodies may also hinder spermatozoon fertilizing capacity.

It is estimated that **5 - 10% of male infertility** is caused by sperm autoimmunity & approximately **10 - 15% of women with unexplained infertility** have circulating sperm antibodies.

The **diagnosis** of antisperm - antibody (ASA) mediated infertility lacks a pathognomonic clinical picture.

However, an ASA should be **suspected** if, the semen analysis shows **sperm agglutination** or **clumping** (i.e. motile spermatozoa stick to each other head-to-head, tail-to-tail or in a mixed way) in the **absence of clinical infection**.

### NOTE :

Sperm antibodies can be present without sperm agglutination; equally, agglutination can be caused by factors others than sperm antibodies.

Following are the **conditions for suspicion** :

- History of **Testis Injury** or **Surgery** with low sperm motility.

- **Increased round cells** i.e.

leucocytes (Repeated Genital Infection).

- Poor results of Post Coital Test.
- Sperm 'Shaking' is observed on Sperm-Cervical Mucus contact test.
- Unexplained infertility.
- Genetic predisposition.

Anti-sperm antibodies (ASAs) in semen belong almost exclusively to two **immunoglobulin classes** : **IgA** & **IgG**. IgM antibodies, because of their larger size, are rarely found in semen. **IgA** antibodies may have **greater clinical importance** than IgG antibodies.

ASA in infertile couples are detected in two ways :

**Direct Test** - Test for antibodies on **sperm membrane**.

**Indirect Test** - Test for antisperm antibodies in **sperm-free fluids**, i.e. Seminal Plasma, blood serum, solubilized cervical mucus.

Numbers of tests are available, but **preferred** tests are **MAR & IBT**. The advantage of **MAR** Test is that it can be **applied directly to fresh, untreated semen samples**. The results can be obtained within few minutes & is quick, simple & repeatable.

## Specimen Preparation

### DIRECT MAR TEST

#### Specimen : SEMEN

- Semen sample is collected with :
  - **Abstinence period** of **2-7 days**.
  - **Ideal collection** through **masturbation** in sterile container.
  - **Non-spermicidal polyurethane semen collection pouch (Sperm Collect™)** can be used when required.
- Semen sample is allowed to liquefy and then well mixed for performing test.

#### Special Instructions :

- **Hyperviscous** semen sample should be **processed** to bring towards normal viscosity. (**Viscosity-CH™** or **Viscosity-BR™** kit can be used)

### INDIRECT MAR TEST

#### Specimen : SERUM

- Collect the **blood sample** in plain bulb.
- Allow **coagulation**.
- **Separate serum**.
- **Incubate** serum at **56°C** for **30min** to inactivate the components.

#### Specimen : CERVICAL MUCUS

- Collect the Cervical Mucus.
- Allow **complete liquefaction**.
- Liquefied cervical mucus sample is **kept** at **56°C** for **30 min** to inactivate the components.

#### Specimen : DONOR SEMEN SAMPLE

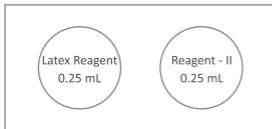
- Collected semen sample should be liquefied with normal viscosity.
- Isolate the **motile** sperms by semen processing (preferred methods - **Swim-up / Swim-down**).
- Adjust the motile sperm **concentration** to **20 - 25 million/mL** & keep it at **37°C** use in Indirect MAR test Step 3.

**NOTE :** To perform Indirect MAR Test specimen needed is **donor semen**.

## Kit Contents

- Latex Reagent : 0.25 mL  
(Blue Colored Latex Particles  
Coated with IgG)
- Reagent - II : 0.25 mL  
(Antihuman IgG Antibody)

### Content Box Diagram :



### Storage Conditions:

- The kit should be stored in dark at 2°C - 8°C after receiving.
- Bring all the reagents to room temperature before use.
- Once opened, store reagents in the fridge protected from light.
- Expiry date is printed on the out side of the box.

## Equipments

### REQUIRED BUT NOT PROVIDED IN KIT

- Microscope
- Controlled Temperature 37°C Dry bath (Sperm Warmer™ / Water bath)
- Semen Analysis Chamber (Sperm Meter™)
- Pipettes Set
- Stopwatch
- Microtip Box
- Glass Slide Tray
- Petri Dish With Glass Rod

## Disposable Materials

### REQUIRED BUT NOT PROVIDED IN KIT

- Hand gloves
- Semen Collection Container
- Non-spermicidal Semen Collection Pouch (Sperm Collect™)
- Microtips
- Pasteur Pipettes
- Test Tubes
- Glass-slides
- Coverslips
- Filter Papers

Degree of agglutination

Parts involved

1. Isolated (<10 sperm/agglutinate, many free sperm)

2. Moderate (10–50 sperm/agglutinate, free sperm)

3. Large (agglutinates >50 sperm, some sperm still free)

4. Gross (all sperm agglutinated, and agglutinates interconnected)

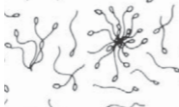
A. Head-to-head



B. Tail-to-tail (heads are seen to be free and move clear of agglutinates)



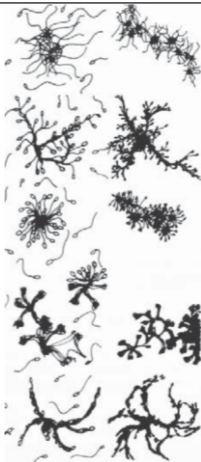
C. Tail-tip-to-tail-tip



D. Mixed (clear head-to-head and tail-to-tail agglutinations)



E. Tangle (heads and tails enmeshed. Heads are not clear of agglutinates as they are in tail-to-tail agglutination)



Reproduced from Rose et al. (1976) by permission of Wiley-Blackwell.

(As per **fifth edition** of **WHO laboratory**

**manual** for examination and processing of **Human Semen**).

## Direct MAR Test

### PROCEDURE :

#### Step 1 :

- **Label** plastic ware & disposable materials with appropriate **Patient ID** & **Sample ID**.
- Bring all the reagents to room temperature.

#### Step 2 : Preparation of Humid Chamber

- Take petri dish.
- Put a wet filter paper (using water to wet) in lower & upper lids of the petri dish.
- Keep two small glass roads in lower lid to hold glass slide horizontally.
- Keep this chamber aside to be used for **Step 5**.

#### Step 3 :

- **Put** down the **glass slide horizontally**.
- **Put** drop of **5  $\mu$ L** fresh liquefied **semen** on glass slide.



#### Step 4 :

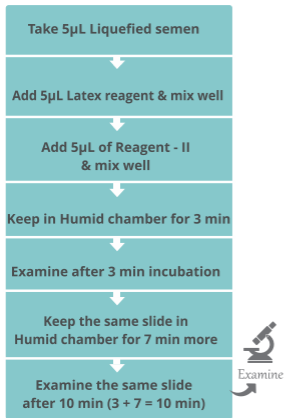
- **Put** drop of **5  $\mu$ L Latex Reagent** (Blue Colored Latex Particles Coated with **IgG**) on the **same area** of glass slide from **step 3**.
- Mix them with the help of sucker tip.



#### Step 5 :

- **Put** drop of **5  $\mu$ L of Reagent - II** (Antihuman IgG Antiserum) on semen / latex particles mix from **step 4**.
- Mix them with the help of a sucker tip.
- Put a coverslip gently to avoid air bubble & **incubate** at room temp. in humid chamber for **3 min**.





## Examination

- Examine the **prepared slide** from **step 5** by using **40x** objective lens preferably phase contrast.
  - Examine **200 – 500 sperms** & **count** the following :
    - Number of **motile sperms**.
    - Number of **motile sperms attached to blue colored latex particle**.
    - Define grade & group of agglutination.
  - If **Grade 4 agglutination** : Examination is complete & proceed to result.
- NOTE** : **No need** to keep the slide in the humid chamber for 7 min more.
- **Keep the slide in humid chamber for 7 min more.**
  - **Total time in humid chamber = 3 + 7 = 10 min**

## AFTER 10 MIN

- \* Re-examine **200 – 500 sperms & count** the following :
  - Number of **motile sperms**.
  - Number of **motile sperms attached to blue colored latex particle**.
  - Define grade & group of agglutination.
- \* Examination is complete & proceed to result.

## Result

	Result After	
	3min	10min
No. of Motile Sperm		
Motile Sperm Attached To Blue Colored Latex Particle		
Grade of sperm agglutination		
Group of sperm agglutination		

### NOTE :

- Compare the result of 3 min & 10 min
- Result of (3 + 7) min can be same as result of 3 min but can never be less.

### Limitations :

- This test provides presumptive quantitative information of sperm.
- This parameter should be analyzed by a specialist.
- The result should be evaluated taking into account all clinical & laboratory findings related to the sample.

## Indirect MAR Test

### PROCEDURE :

**Step 1 :** • **Label** plastic ware & disposable materials with appropriate **Patient ID & Sample ID.**

- Bring all the reagents to room temp.

**Step 2 :** Dilute **serum / cervical mucus** 1:5 ( i.e. **20  $\mu$ L** of serum/cervical mucus & **80  $\mu$ L** of Sperm Washing Media) & mix well.

**Step 3 :** Take **50  $\mu$ L** of diluted serum/cervical mucus from **step 2** & **50  $\mu$ L** of **prepared donor semen** in microtube & mix well.

**Step 4 :** Incubate at **37°C** for **60 min.**

**Step 5 : Preparation of Humid Chamber**

- Take petri plate
- Put a wet filter paper (using water to wet) in lower & upper lids of the petri plate.
- Keep two small glass roads in lower lid to hold glass slide horizontally.

- Keep this chamber aside to be used for Step 8.

**Step 6 :** After **60 min**, take **5  $\mu$ L** of specimen from **step 4** & drop on glass slide.

**Step 7 :**

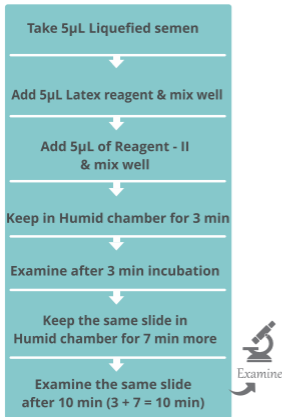
- **Put** a drop of **5  $\mu$ L** of **Latex Reagent** (Blue Colored Latex Particles Coated with Antihuman IgG Antibody) on the **same area** of glass slide from step 6.
- Mix them with the help of a sucker tip.

**Step 8 :**

- Put a drop of **5  $\mu$ L** of **Reagent - II** (Antihuman IgG Antibody) on the **same area** of glass slide from step 6.
- Mix them with the help of a sucker tip.
- Put the coverslip gently to avoid air bubble & **incubate** at room temp. in humid chamber for **3 min.**

## Quick Glance

Incubated sample (Step 4)  
(50  $\mu$ L Prepared Serum/Cervical Mucus &  
50  $\mu$ L Prepared Donor Semen Sample)



## Examination & Result :

- Examine the prepared slide from **step 8** by using **40x** objective lens preferably phase contrast.
- Follow the procedure for **examination & result** given at page no. **14 , 15 & 16**

## Limitations :

- This test provides presumptive quantitative information of sperm.
- This parameter should be analyzed by a specialist.
- The result should be evaluated taking into account all clinical & laboratory findings related to the same sample.

## Precautions

- All patient samples & reagents should be treated as potentially infectious & the user must wear protective gloves, eye protection & laboratory coats when performing the test.
- The kit should be discarded in a proper biohazard container after testing.
- Do not eat, drink or smoke in the area where specimens & kit reagents are handled.
- Do not use beyond the expiration date which appears on the package label.
- It is recommended to use of gloves & face mask.

## Safety & Environment

- Do not release the products used into the environment. Follow centre guidelines for the storage & disposal of toxic substances.
- Biological samples must be handled as potentially infectious.

## Description of Symbols



consult instructions of use



product reference



lot number



use by



manufacturer



health surveillance device  
for in-vitro diagnostic



contains sufficient for 'n' tests



temperature limitation



keep dry



CE mark (Conformité Européene)

## Accreditations & Registered Certificates

- **ISO 13485 : 2003** Certified
- **CE** Accredited
- **GMDN** Registered
- **US FDA** Registered

For more information & procedure videos



[www.spermprocessor.com/sft-antisperm-antibody.html](http://www.spermprocessor.com/sft-antisperm-antibody.html)

IgG-Direct ▶  
procedure video



<https://www.youtube.com/watch?v=uzsLYIpEOe>

IgG-Indirect ▶  
procedure video



<https://www.youtube.com/watch?v=edF4Eak7JZM>

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