Antisperm-Antibody (IgA)
MAR Test
(mixed antiglobulin reaction)

Red Colored Coated Latex Particles

REF SP/SFT/ASA-007-B

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Turnaround time for test
- IgG Direct: 15min
- IgG Indirect: 90min

Store at: 2°C - 8°C after receiving
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 spermatozoaon 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.

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.

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.
**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 30 min to inactivate the components.

**Specimen : CERVICAL MUCUS**
- Collect the Cervical Mucous.
- 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 (Red Colored Latex Particles Coated with IgA)

Content Box Diagram:

- Latex Reagent: 0.25 mL

Equipment

- 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

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 outside of the box.

Disposable Materials

- Hand gloves
- Semen Collection Container
- Non-spermicidal Semen Collection Pouch (Sperm Collect™)
- Microtips
- Pasteur Pipettes
- Test Tubes
- Glass-slides
- Coverslips
- Filter Papers
<table>
<thead>
<tr>
<th>Degree of agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parts involved</strong></td>
</tr>
<tr>
<td>1. Isolated (&lt;10 sperm/agglutinate, many free sperm)</td>
</tr>
<tr>
<td>2. Moderate (10–50 sperm/agglutinate, free sperm)</td>
</tr>
<tr>
<td>3. Large (agglutinates &gt;50 sperm, some sperm still free)</td>
</tr>
<tr>
<td>4. Gross (all sperm agglutinated, and agglutinates interconnected)</td>
</tr>
</tbody>
</table>

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 μL** fresh liquefied **semen** on glass slide.

Step 4:
- **Put** drop of **5 μL Latex Reagent** (Red Colored Latex Particles Coated with Antihuman IgA Antibody) on the **same area** of glass slide from **step 3**.
- Mix them with the help of sucker tip.

Step 5:
- Put a coverslip gently to avoid air bubble & **incubate** at room temp. in humid chamber for **3 min**.
Quick Glance

- Take 5µL Liquefied semen
- Add 5µL Latex reagent & mix well
- Keep in Humid chamber for 3 min
- Examine after 3 min incubation
- Keep the same slide in Humid chamber for 7 min more
- Examine the same slide after 10 min (3 + 7 = 10 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 red 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 red colored latex particle**.
  - Define grade & group of agglutination.

* Examination is complete & proceed to result.

<table>
<thead>
<tr>
<th></th>
<th>Result After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3min</td>
</tr>
<tr>
<td>No. of Motile Sperm</td>
<td></td>
</tr>
<tr>
<td>Motile Sperm Attached To Red Colored Latex Particle</td>
<td></td>
</tr>
<tr>
<td>Grade of sperm agglutination</td>
<td></td>
</tr>
<tr>
<td>Group of sperm agglutination</td>
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</tbody>
</table>

**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 µL of serum/cervical mucus & 80 µL of Sperm Washing Media) & mix well.

**Step 3:**
Take 50 µL of diluted serum/cervical mucus from **step 2** & 50 µL 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 µL of specimen from step 4 & drop on glass slide.

**Step 7:**
• **Put** a drop of 5 µL of **Latex Reagent** (Red 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 the coverslip gently to avoid air bubble & **incubate** at room temp. in humid chamber for 3 min.
Quick Glance

Incubated sample (Step 4)
(50 µL Prepared Serum/Cervical Mucus & 50 µL 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.

Description of Symbols

- 
- consult instructions of use
- 
- product reference
- 
- lot number
- 
- use by
- 
- manufacturer
- IVD
- health surveillance device for in-vitro diagnostic
- Σ
- contains sufficient for ‘n’ tests
- 
- temperature limitation
- 
- keep dry
- 

Safety & Environment

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

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

For more information & procedure videos


IgG-Direct procedure video
https://www.youtube.com/watch?v=uzsLYIpEOoE

IgG-Indirect procedure video
https://www.youtube.com/watch?v=edF4Eak7JZM

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