Menicon Life Science

Microfluidic Device for Isolation of Motile Sperm

QUALIS クオリス SPERM SORTER

Instruction Manual

For research only in countries or regions other than Japan

Menicon

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Thank you for purchasing Sperm Sorter Qualis from Menicon Co., Ltd.
Please read all instructions carefully before starting your operation and keep this instruction manual handy for easy reference.

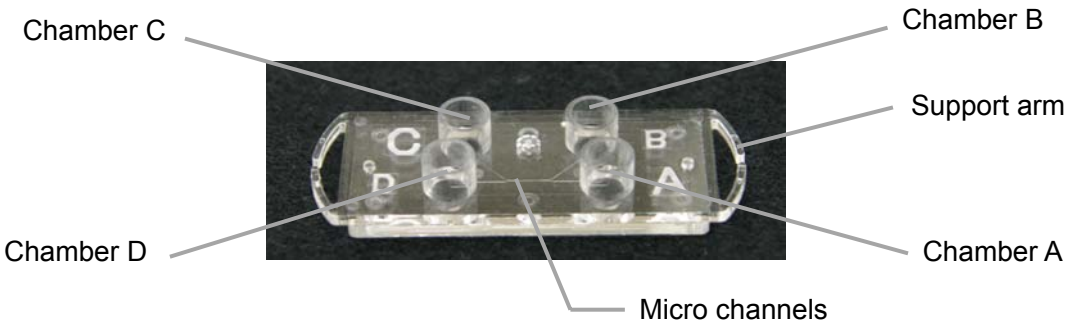
INTENDED USE

Sperm Sorter Qualis is intended to be used for the isolation of motile sperms from semen.

PRECAUTIONS

- Do not use Sperm Sorter Qualis for any purpose other than motile sperm isolation.
- Do not use or operate Sperm Sorter Qualis if it shows signs of damage, crack or bending, or if package broken.
- To prevent any damage to the Sperm Sorter Qualis, do not apply any excess weight on the device. Avoid exposure to excessive heat during storage.
- To prevent contamination, use the device immediately after opening its package.
- Sperm Sorter Qualis is a disposable device intended to be used once. It cannot be reused.
- Disposal of Sperm Sorter Qualis should follow specific laws or regulations of your institute.

PARTS NAMES



NECESSARY INSTRUMENTS

- φ60mm dish (BD Cat#351007, 353002, 353802, 353652) or equivalent
- Micropipette (Nichipet ExCE200, Nichiryo) or equivalent
- Micropipette (Nichipet Ex CE10, Nichiryo) or equivalent
- Micropipette chips qualified for each micropipette
- Phase contrast inverted microscope or stereomicroscope for 40-100 times observation
- Nippro foreign body removal filter (pore size 20 μm) or equivalent
- Nippro sperm processing chip or equivalent
- Lock syringe (Thermo Cat#SS-05LZ) or equivalent

PROCEDURES FOR USE

A) Preparation of sperm sorting medium

Recommended sperm sorting media by Menicon

Medium name	Company	Serum substitute supplement ★
Modified HTF Medium-HEPES	Irvine Scientific	Necessary
Sperm washing Medium		Not necessary
global w/HEPES	Life Global	Necessary
HTF w/HEPES		
HTF Xtra w/HEPES		
G-MOPS™	Vitrolife	Necessary
G-MOPS™ PLUS		Not necesary

★Serum substitute supplement (Irvine Scientific)

1. Prepare sperm sorting medium.
2. Keep sperm sorting medium at approximately 37℃ before use.

B) Preparation of sperm suspension

1. Allow semen to liquefy after ejaculation.
2. Dilute liquefied semen with sperm sorting medium at the ratio of 1:1, mix this sperm suspension completely by gentle pipetting. 500 µl sperm suspension is enough for sperm sorting.
3. Filter the sperm suspension by Nippro foreign body removal filter (pore size 20µm). This step is recommended to prevent foreign bodies to block the micro channel.
4. Keep sperm suspension warm at approximately 37 ℃ before sperm sorting.

C) Preparation of Sperm Sorter Qualis

1. Fix Sperm Sorter Qualis in a ϕ60 mm dish with support arm until no any relative movements between Sperm Sorter Qualis and dish (Fig 2).

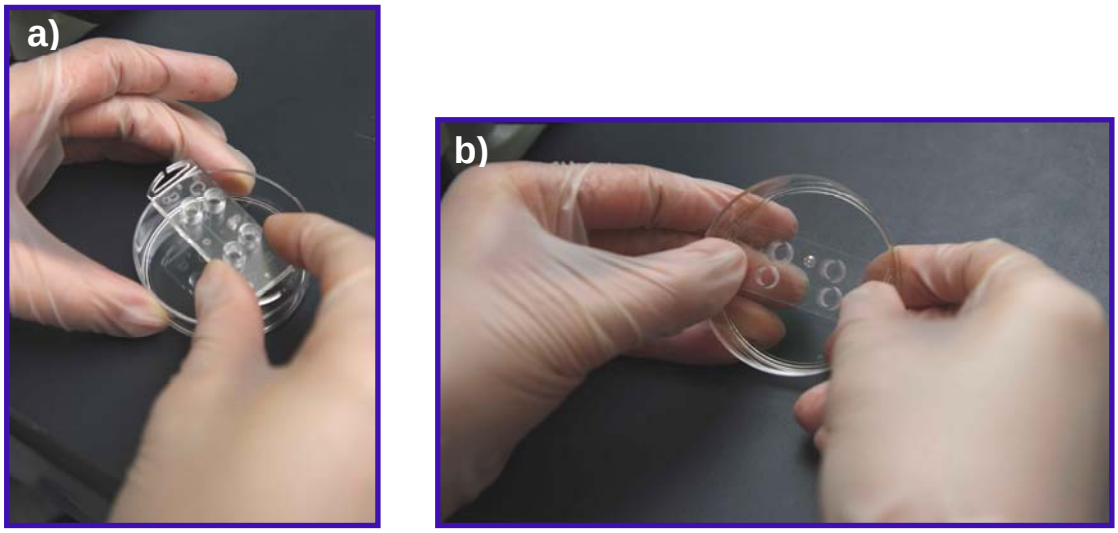


Fig 2. Fix Sperm Sorter Qualis in a ϕ60 mm dish. a). Place diagonally Sperm Sorter Qualis in a ϕ60 mm dish, b). push the edges of Sperm Sorter Qualis with two thumbs to allow sperm sorter bottom to be attached with the dish completely.

2. Soak chambers and micro channels

This step is necessary to increase the hydrophilicity of Sperm Sorter Qualis.

a. Load 100 µL sperm sorting medium into Chamber A (close to ☆) (Fig 3.) . Confirm that micro channels are full of loaded sperm sorting medium [note 1] and sperm sorting medium has arrived in chambers B, C and D.

Note 1：Bubbles rarely occur in the micro channels.

【In case tiny bubbles do not block the flow】

Bubbles won't affect sperm sorting, please proceed to next step.

【In case bubbles block the flow】

- Bubbles might disappear when being allowed to stand for a while.
- Push bubble out slowly by pipetting sperm sorting medium from chamber A（close to ☆, Fig 3）.
- Change to a new Sperm Sorter Qualis if bubbles are not removed.

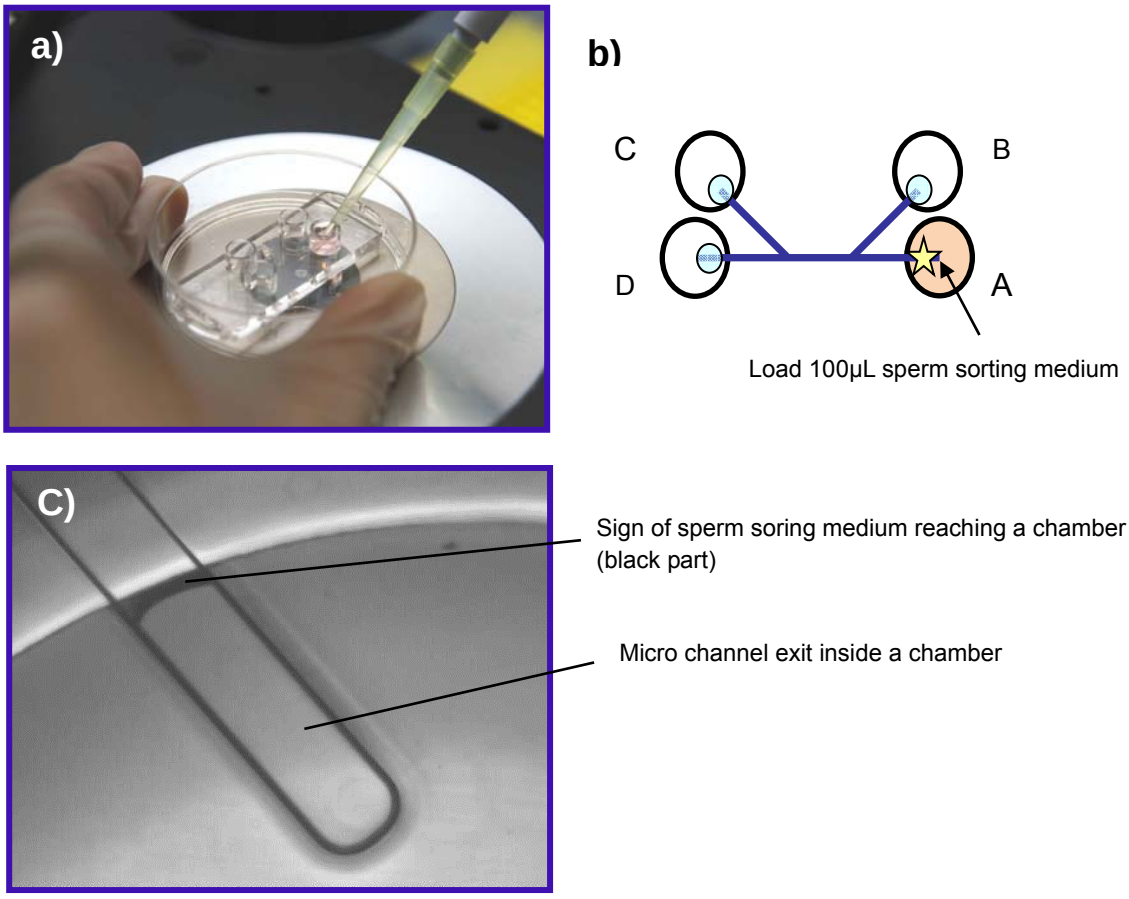


Fig 3. Load sperm sorting medium into Chamber A. a, b) Load sperm sorting medium to the area of micro channel exit (☆). c) Confirm sperm sorting medium arrives in Chambers B, C and D under the microscope.

b. Load 100 µL sperm sorting medium into Chamber B, C and D respectively (Fig 4).

c. Pull out all the sperm sorting medium from all chambers.

Fig 4. Load sperm sorting medium into chambers. Change Sperm Sorter Qualis direction for easy operation.

D) Sperm sorting procedure

1. Load sperm sorting medium and sperm suspension

   a. Load 20 µL sperm sorting medium into Chambers C and D (close to ☆, Fig 5) respectively.
   b. Load 100 µl sperm sorting medium into Chamber B (close to ☆, Fig 5)
   c. Load 65 µl sperm suspension into Chamber A.

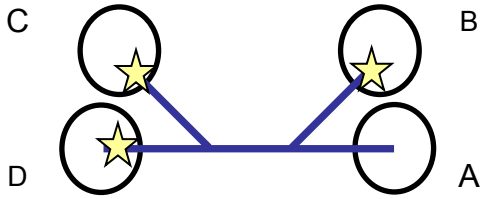


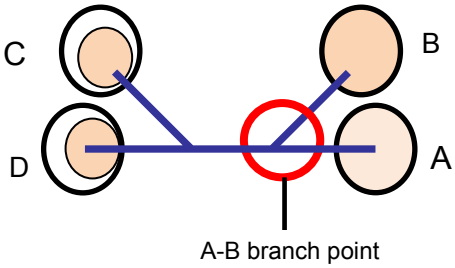
Fig 5. Areas to load sperm sorting medium in Chambers B, C and D.

「laminar flow」 is a set of streamline flows of fluid in parallel layers, whereas "turbulent flow" is a set of rough flows with cross and mixed streamlines.

2. Optimize laminar flow in channels

   a. Observe the branch point of laminar flow from Chamber A and B under the microscope at forty times (Fig 6).

   Pull out sperm sorting medium from Chamber B to adjust the width of laminar flow from Chamber A to reach 40% of the overall width of the micro channel. It is recommended to pull out a few microliters for each operation and to repeat several times (Fig 7). Refer to **ADJUSTMENT OF LAMINAR FLOW** on page 8.



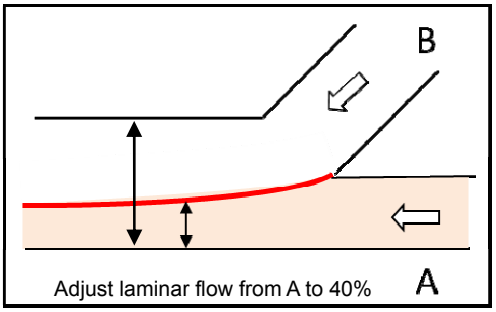


Fig 6. Observe A-B branch point under the microscope. Check the width of laminar flow from Chamber A.

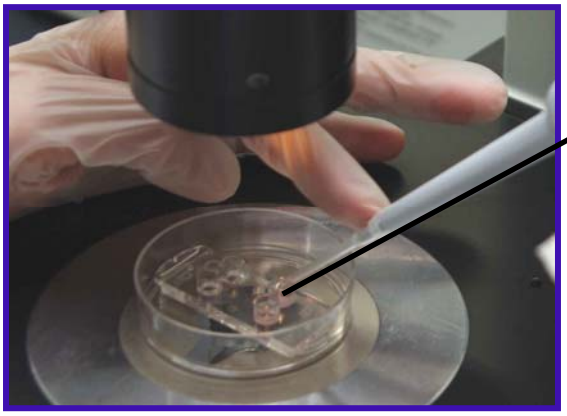
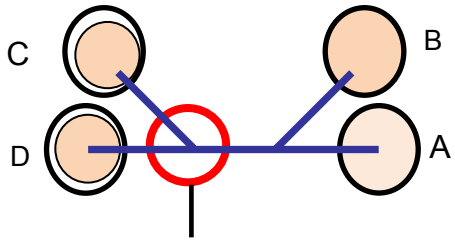


Fig 7. Pull out sperm sorting medium from Chamber B. Pull out a few microliters from Chamber B for several times while confirming the width of laminar flow from Chamber A. Usually approximately 15 µL in total will be pulled out to reach the optimized width of the laminar flow.

b. Observe the branch point of laminar flow to Chamber C and D under the microscope at forty times (Fig 8).

To confirm laminar flow from Chamber A has not arrived in Chamber C.



C-D branch point

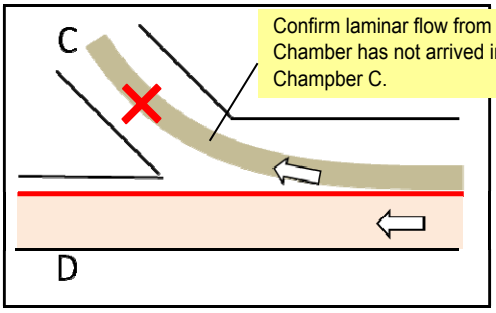


Fig 8. Observe C-D branch point under the microscope. Confirm laminar flow from Chamber A has not arrived in Chamber C.

c. Motile sperm sorting

Cover the Sperm Sorter Qualis with ϕ 60 mm dish cover to prevent from drying, bacteria or dust contamination. Allow Sperm Sorter Qualis to stand for 10 minutes [Note 2]. Label either ID number or bar code on the dish cover.

Note 2 : In case that semen analysis shows extremely low sperm concentration and poor motility, sperm sorting process could be extended. We have confirmed a stable laminar flow for 30 minutes.

Fig 9. Photo of Sperm Sorter Qualis in sorting process

E) Extraction of isolated sperms

1. Observe C-D branch point under the microscope, confirm laminar flow from Chamber A has not arrived in Chamber C.

2. Extract[Note 3] the overall volume of medium in Chamber C. Because sperm tends to reservoir to the areas close to channel exit and chamber wall, please specifically extract from those areas.

> Note 3：Approximately 25 μL medium could be extracted from Chamber C. This procedure by pipette should be completed once if possible. Decrease in medium volume in Chamber C might cause collapse of the laminar flow, resulting in a liquid flow from Chamber A into Chamber C.

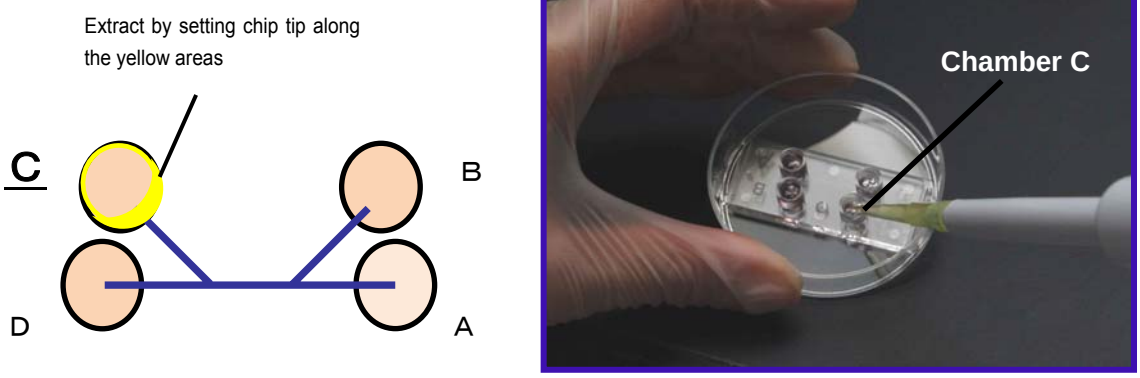


Fig 10. Extract isolated sperms from Chamber C. As shown in the right photo, change Sperm Sorter Qualis direction for easy operation. Tilt the dish, set chip tip along with the wall to extract overall volume of Chamber C. The chip showed in the photo is for 100 μL use. A fine-tipped chip is recommended.

ADJUSTMENT OF LAMINAR FLOW

A) Laminar flow from Chamber A is narrow.

Pull out a few microliters of sperm sorting medium from Chamber B for several times until the width of laminar flow from Chamber A reaches 40% of the whole width of micro channel (Fig 11).

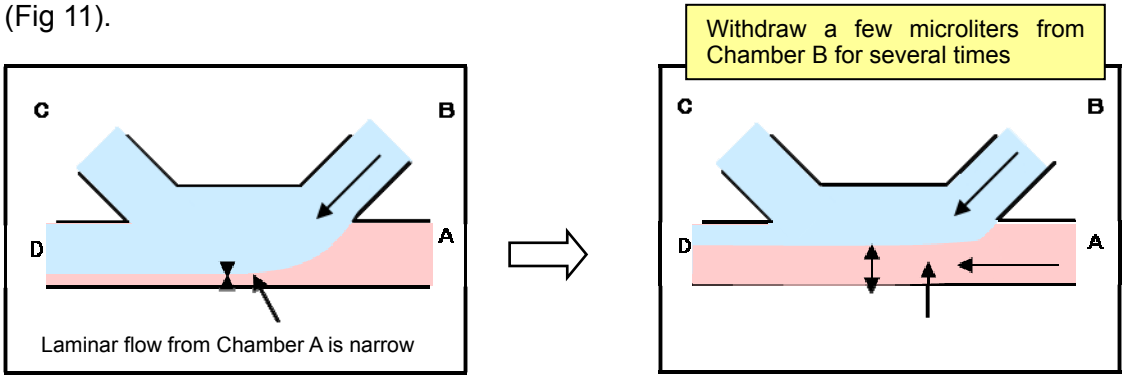


Fig 11.

B) **Laminar flow from Chamber A is wide.**

Add a few microliters of sperm sorting medium into Chamber B for several times until the width of laminar flow from Chamber A reaches 40% of the whole width of micro channel (Fig 12).

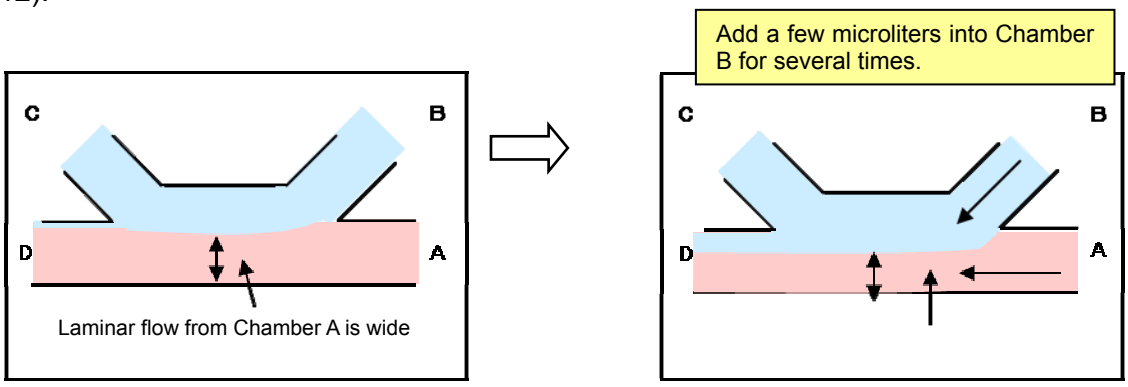


Fig 12.

C) **Laminar flow from Chamber A arrives in Chamber C.**

In case of Fig 13, stop the sperm sorting procedure and renew the Sperm Sorter Qualis .

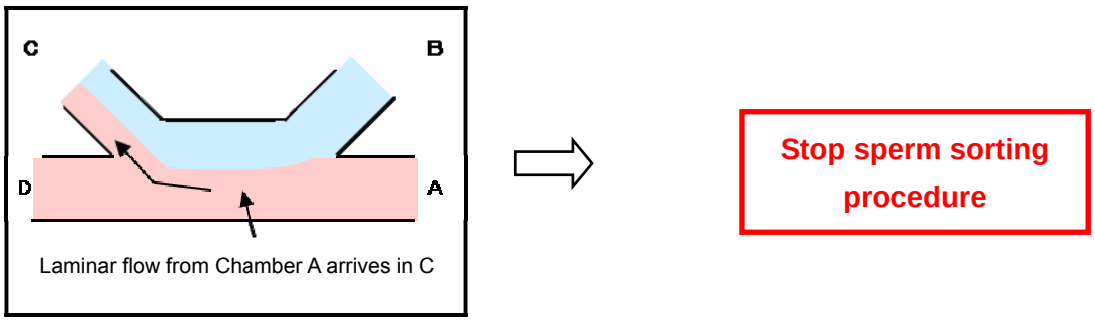


Fig 13.

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