QwikCheck® DFI KIT Instructions for Use

Introduction and Intended Use

The QwikCheck DFI Kit is an in-vitro use diagnostic kit based on the Sperm Chromatin Dispersion (SCD) method and intended for clinical laboratory use. The kit simplifies the slide preparation by providing reagents, agarose slides, and pre-measured agarose vials to enable the lab to prepare ten slides for assessing sperm halos and reporting the DNA Fragmentation Index (DFI) of the semen sample. Once the slide is prepared following the procedure outlined below, sperm cells can be assessed based on laboratory protocols using a microscope or the SQA-Vision based on WHO 6th guidelines.

Storage Conditions

Store kit at 2°C-8°C (36°F-46°F) and refer to box labeling for open and closed vial shelf life. Expiration dates assume the QwikCheck DFI Kit components are stored in their original containers and tightly capped to prevent evaporation.

Precautions:

- **Wear a laboratory coat, mask, and protective gloves.**
- **Use of a fume hood is recommended while preparing the Lys Plus* solution and the slide in Steps 6-7.**
- **When first opening the bottles, remove the cap separator. Then re-cap the bottle while pressing down to puncture the bottle dropper.**
- **The patient samples and reagents should be treated as potential hazards.**

Materials and Methods for Use

QwikCheck DFI Kit Components for 10 Tests

	Reagents and Materials	#	Content	Use
1	Diluent	1	10 mL / wide-mouth bottle	Semen sample dilution, see table below
2	Low Gelling Agarose	10	0.1 mL in 0.5 mL vial	To embed sperm
3	Agarose Coated Slides	10	Agarose Coated Slides	To provide slides with the underlying matrix
4	Coverslips	12	Coverslips 40 mm X 24mm	To place over the agarose drops to form a rectangular film
5	Lys 1 Solution	1	10 mL / wide-mouth bottle	Sperm denaturation
6	Lys 2 Powder	10	30 mg in 2 mL amber vial	Pre-measured powder. Mix with Lys 2 solution to obtain Lys Plus*
7	Lys 2 Solution	1	10 mL / wide-mouth bottle	Solution to mix with Lys 2 powder to obtain Lys Plus*
8	Washing solution	1	15 mL / dropper bottle	Wash Lys reagents
9	Fixative	1	10 mL / dropper bottle	Sperm fixation
10	Giemsa A	1	10 mL / dropper bottle	Sperm staining
11	Giemsa B	1	10 mL / dropper bottle	Sperm staining

Checklist of equipment and materials required but not provided in the kit:

- SQA Vision / Microscope and Concentration Assessment Chamber / Fume Hood / Refrigerator (2°C -8°C / 35.6°F-46.4°F)
- Centrifuge / Centrifuge tubes / Micropipette & Tips / At least 1.5 ml vial for dilution / Stopwatch / Staining Tray
- Dry bath of both 80-90°C (176-194°F) and 37°C (98.6°F) / Filter Paper / Distilled Water in Squeeze Bottles

1. Prepare Ahead:

1. **Agarose Pre-Coated Glass Slides (3):** Bring to room temperature at least 10 minutes before use. Touch only the frosted region of the slide when removing from the box and during the entire preparation / staining process. **Make sure the MES logo is right side up for testing.**
2. **Make Lys Plus*:** Add 1 mL of Lys 2 solution (7) to one amber vial of Lys 2 powder (6). Close the lid and mix well by vortex or by manually rolling the vial between palms. **NOTE: Use only one amber vial per test. Discard any leftovers and do not re-use or store them as the pH will change.**
3. **Assess Sperm Concentration:** Check sperm concentration after sample liquefaction using the laboratory method. When using SQA-Vision to assess sperm concentration, use the setting option: Scan for Debris On All Samples. DO NOT pre-dilute the sample with any other reagent/media. **NOTE: If QwikCheck Liquefaction is used for sample liquefaction, complete DFI staining within 1 hour of sample liquefaction.**
4. **Sample Dilution:** To prevent overlapping spermatozoa, dilute the sample based on the native sample sperm concentration as shown in the following table. Use only the DFI Kit Diluent supplied in the kit.

Sample Dilution Table

Sperm Concentration (M/mL)	Dilution Ratio	Volume	
		Sample (µL)	Diluent (µL)
10-25	1:2	100	100
25-50	1:4	100	300
50-70	1:6	100	500
70-95	1:7	100	600
95-140	1:10	100	900
Above 140	1:20	50	950

Low Volume/Low Quality Samples

If Sample volume is >40 µl and <100 µl and Sperm Concentration is ≥ 10m/mL	• Determine Dilution Volume using this formula: (CONC m/mL X semen volume ml) ÷ 10 – VOL ml
If Sample Volume is >500 µl and Sperm Concentration is <10m/mL	• Centrifuge sample, decant supernatant, and resuspend the pellet with DFI Kit Diluent as follows: Resuspension Volume ml = (CONC m/mL x semen volume ml) ÷ 10.
If Sample volume is >40 µl and <100 µl and Sperm Concentration is <10m/mL	• The sample is oligozoospermic and cannot be run.

2. Slide Preparation for Testing

1. **Melt Low Gelling Agarose (@80-90°C / 176-194°F; 2 minutes):**
   Use 1 vial of agarose (2) per test. Melt the agarose in a dry bath pre-heated to 90°C (194°F) for 2 minutes. Repeat if not melted.

2. **Incubate Low Gelling Agarose (@37°C / 98.6°F; 5 minutes):**
   Transfer the agarose vial to a dry bath pre-heated to 37°C (98.6°F) and incubate for 5 minutes.
   NOTE: High temperatures can damage sperm cells.

3. **Mix 40µL Diluted Semen and Melted Agarose (@37°C / 98.6°F):**
   Add 40µL of the diluted semen to the melted agarose vial and mix well by gently pipetting 10 times, to avoid creating bubbles.

4. **Prepare Agarose Coated Slide with the Semen/Agarose Mixture (@20-25°C / 68-77°F):**
   Without scratching the slide, place two 20 µL drops of the Semen/Agarose Mixture, one drop below the other 10mm apart, as shown in Figure 1. Cover with a coverslip (24mmx40mm).
   NOTE: This process is very temperature sensitive. Do not place the Agarose Coated Slide on metal surfaces.

Figure 1. Semen/Agarose Mixture drop location simulation (in red

5. **Solidify the Agarose (@ 2-8°C / 36-46°F; 5 minutes):**
   Refrigerate the slide on a flat surface for 5 minutes @ 2°C-8°C (36°F-46°F). DO NOT place the slide in the freezer.

6. **Remove the Coverslip (controlled room temperature from this step on):**
   After 5 minutes, take out the slide from the refrigerator. Gently remove the coverslip in a sliding motion without breaking the agarose film embedding the spermatozoa, as shown in Figure 2.

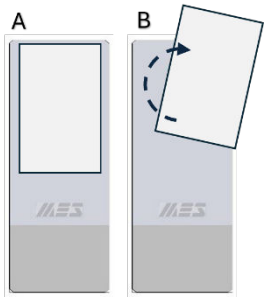

Figure 2. Coverslip Removal.
A. coated slide after incubation.
B. coverslip removal by sliding

7. **Lys 1 (2.5 minutes; 1000 µL):**
   Place the staining tray within the fume hood. Pipette 1000 µL of Lys 1 (5) on the slide. Ensure the entire agarose area is covered.
   Wait 2.5 minutes.

8. **Lys Plus* (10 minutes; 1000 µL):**
   Decant the Lys 1 solution. Pipette 1000 µL of freshly prepared Lys Plus* solution on the slide. Ensure the entire agarose area is covered. Wait 10 minutes.
   NOTE: When pipetting, ensure that the solution is homogenous.

9. **Washing Solution (2 minutes; ~20 drops):**
   Decant the Lys Plus* solution, then flood the agarose area with the Washing Solution (8) by placing at least 20 drops.
   Wait 2 minutes.

10. **Fixative (4 minutes; 4 drops):**
    Decant the washing solution. Place at least 4 drops of the Fixative (9) on the agarose film area of the slide. Cover the whole agarose film area. Leave it on for 4 minutes.
    Decant Fixative from the slide: Decant any residual Fixative and then wipe the **backside** of the slide. Air dry the slide for 5-15 minutes to completely dry and evaporate any remaining fixative (increasing airflow within the fume hood can hasten the evaporation).

11. **Stain with Giemsa A solution (1 minute; 6 drops):**
    Once the slide is verified to be completely dry, **reduce** the airflow within the fume hood to decrease the evaporation of the stain in this step. Place at least 6 drops of Giemsa A (10) stain on the agarose film area of the slide. Cover the whole agarose film area. Leave it on for 1 minute.
    DO NOT decant the Giemsa A solution.

12. **Stain with Giemsa B solution (2 minutes; 9 drops):**
    Place at least 9 drops of Giemsa B stain (11) on top of the Giemsa A solution. Cover the whole agarose film area. Make sure that the two solutions mix well and form an even layer, without dripping off the slide.
    Leave the solutions on the slide for 2 minutes until a **metallic sheen** can be seen forming on the top of the solution.

13. **Decant the Stain solutions and Wash with Distilled Water (20-60 seconds):**
    Tip the slide onto a staining tray or sink. Using Distilled Water in a squeeze bottle with a tip, gently wash the slide from the frosted area of the slide downward for 60 seconds at most. A light pink tinge will remain on the agarose film area of the slide.
    NOTE: The water stream should touch only the frosted part of the slide and flow downward to the agarose area. DO NOT let the water stream touch the agarose area to avoid film damage.

14. **Air Dry the Slide (5-15 minutes):**
    Wipe the backside of the slide, do not touch the agarose film area at the front-side of the slide, and Air Dry until visibly dry and no water droplets remain.

3. Classify Halos

Classify Halos: Assess a minimum of 200 spermatozoa and classify halos according to WHO 6th Edition or laboratory protocols using the SQA-Vision DNA Fragmentation Assessment tool or under a microscope at 1000X magnification (with immersion oil).

4. DFI Kit Limitations

DFI Kit provides information on spermatozoa DNA fragmentation. This test alone does not indicate male fertility status that should be evaluated by specialist using comprehensive semen analysis data and clinical assessment.

NOTE: DNA Fragmentation Assessment tool will only mark cells per WHO 6th Edition semen analysis protocols.