

Training Video



1 Sperm Concentration

Assess Sperm Concentration (Using SQA-Vision or Microscope)



Dilute or Centrifuge the Sample to Reach Optimal Range (per table)

2 Prepare Lys Plus Solution

Mix **1 mL** of Lys 2 Solution with Lys 2 Powder to Create "Lys Plus"*

3 Prepare the Agarose

Melt Agarose (90°C for **2 Minutes**)



Incubate Agarose (37°C for **5 Minutes**)



Mix **40 μL** of Prepared Semen with the Melted Agarose and Mix well

4 Agarose Coated Slide

Place 2 x 20 µL Drops of Semen Mixture on Agarose Coated Slide (10mm Apart in the Viewable Area)



Cover the Slide with a Coverslip (24mm x 40mm)



Refrigerate the Slide (2°-8°C for **5 Minutes**)



Carefully pull out the Coverslip with a slide motion parallel to the slide

5 Slide Washing & Staining

Lys 1 Solution: Add **1 mL** on the slide (Wait **2.5 Minutes**) Decant Lys 1



Lys Plus Solution: Add 1 mL on the slide
(Wait 10 Minutes)



Decant the Slide and then Wash with

Washing Solution

(Flood the slide for 2 Minutes)



Fixative: Add 4 Drops to the Slide (Wait 4 Minutes)



Decant the Wipe the Backside Dry (Do Not Touch Front) Air Dry for 5-15 Minutes



Giemsa A: Add 6 Drops (Wait 1 Minute) Do Not Decant



Giemsa B: Add 9 Drops (Wait 2 Minute)



Decant, then **Wash** with **Distilled Water** using a Squeeze Bottle*
(60 Seconds Maximum)

*The Water stream should touch only the frosted part of the slide and flow downward over the slide



Decant the Slide and Wipe the Backside Dry (Do Not Touch Front) **Air Dry** for 5-15 Minutes

6 Classify Halos

Assess Sperms Using the SQA-Vision or Microscope @1000 Magnification