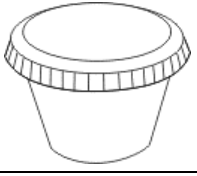

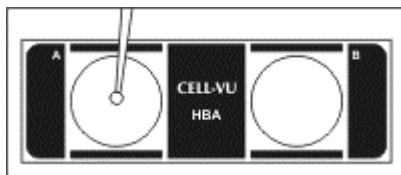
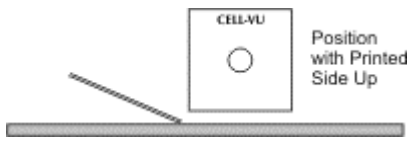

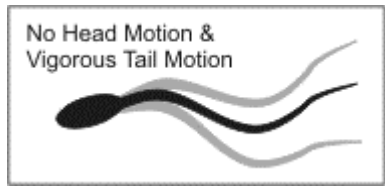
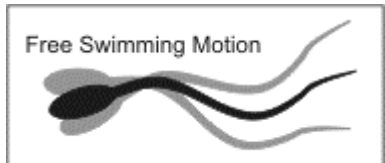


HBA: Sperm-Hyaluronan Binding Assay

Quick Start Reference Sheet

SPECIMEN COLLECTION AND PREPARATION		
1	Collect semen into a clean, dry container by masturbation, preferably following 2-3 days of abstinence.	
2	Maintain the sample at room temperature (18-28° C) for 30 to 60 minutes to allow it to liquefy. If the sample does not liquefy after 60 minutes, dilute an aliquot of the specimen with an equal volume of a sperm dilution medium such as human tubal fluid (HTF), and mix by drawing the mixture up and down in the pipet tip.	
3	Test the semen specimen within 3 hours of collection.	

STEP-BY-STEP TEST PROCEDURE		
1	Perform the test at room temperature, 20-30°C . Do not exceed 30°C.	
2	Mix the sample and pipette 7-10 µL of sample near the center of the chamber.	
3	Place the CELL-VU gridded cover slip (printed side up) over the chamber, taking care to avoid air bubble formation. This may be accomplished by slowly lowering the coverslip at an angle.	
4	Let the slide sit at room temperature for at least 10 minutes, but not more than 20 minutes .	
5	Count the number of bound, motile sperm and unbound, motile sperm (see below) in the same number of grid squares . <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>Bound, Motile Sperm show:</p>  </div> <div style="text-align: center;"> <p>Unbound, Motile Sperm show:</p>  <p>(continued on reverse)</p> </div> </div>	
6	<ol style="list-style-type: none"> Perform a quick visual scan of the chamber to determine the most common motile sperm type, bound or unbound, and count this type first. Count until at least 100 cells have been reached, or all 100 grids have been counted. Record both the number of cells counted and the number of grids counted. Repeat the count in the same number of grid squares for the remaining type of motile sperm, and record this number. Add these numbers to obtain the total motile sperm. 	
7	Calculate the percent hyaluronan-binding motile sperm as follows: $\%Bound = 100 \times Bound\ Motile / Total\ Motile$	