The HBA® Sperm-Hyaluronan Binding Assay

Proprietary and Established Names: HBA® Sperm-Hyaluronan Binding Assay

Indications for Use:
The HBA® Sperm-Hyaluronan Binding Assay is intended to:

1. As a component of the standard analysis of semen in the diagnosis of suspected male infertility.
2. As a component of a clinical test to determine the proper course of IVF treatment of infertility.

Intended Use:
The HBA Assay is a qualitative assay for the maturity of sperm in a fresh semen sample. It is intended to provide additional information to professionals for the evaluation of an infertile couple. It is not intended to be a single diagnostic indicator of potential fertility. The assay is based on the ability of mature, but not immature, sperm to bind to hyaluronan, the main macromolecular component of the cumulus oophorus and a component of human follicular fluid.

Summary:
In natural sperm maturation, sperm bind to hyaluronan, the main component of the cumulus oophorus matrix; immature sperm do not bind (3, 9, 13, 14, 19, 21).

Mature sperm also bind to hyaluronan chemically attached to a support (9, 10) such as the hyaluronan-coated glass slides that make up the HBA® kit. Viewed in the microscope, bound sperm are differentiated from unbound sperm by their beating tails which make no progressive movements. Sperm unable to bind to hyaluronan share many aspects of immaturity: they retain cytoplasm and histones (9), and the proportion of mature sperm indicates the maturity of sperm in a semen sample.

The HBA® Slide:
The HBA® slide contains two identical assay chambers. Each chamber has a thin layer of hyaluronan bound to a glass cover slip placebo. The chamber depth is uniform and will be between 14-24 microns. By counting the numbers of motile bound and unbound sperm in each assay area, the proportion of binders in the motile sperm population can be calculated.

The Physiological Basis for the Assay:
Hyaluronan binding indicates the successful completion of spermiogenic events including: mitotic segregation of one complete haploid chromosomal complement; stabilization of the chromosomes by subsequent interactions with histones; protection against nuclear damage; extrusion of cytoplasm; synthesis of midpiece, tail, and acrosome; and remodeling of the plasma membrane to incorporate hyaluronan and zona binding receptors. The success of these events is directly linked to the timing and expression of a critical chaperone protein, HspA2, during spermiogenesis (2, 8). The chaperone is a component of the synaptosomal complex (1) and like other chaperones in the Hsp70 family, it fosters intracellular transport and membrane remodeling. In the absence of the chaperone, deficiencies in sperm maturation may occur, including deficits in the critical functions listed above and in hyaluronan binding.

Specimen Collection and Preparation:
Semen should be obtained by masturbation, preferably following 2-3 days of abstinence. It should be collected in a clean, dry container and kept at room temperature (20-28°C) for 30 minutes to liquefy. A specimen is considered liquefied if it can be pipetted into a pipette and discharged cleanly, i.e., without lumps blocking the tip. If the specimen cannot be cleanly pipetted, wait an additional 30 minutes and test again. If it still appears non-liquefied, dilute an aliquot of semen 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 pipette tip. This will produce a specimen that can be applied to the HBA® slide.

Perform the assay on the semen specimens within 3 hours of collection.

Warnings and Precautions:
1. Sperm is a hazardous material and must be handled and disposed of using normal precautions for infectious, biohazardous materials. In particular, wear gloves, eye protection and a laboratory coat. Disinfect all semen waste and dispose of it in a properly labeled biohazard container.
2. Do not use HBA® slides for selecting sperm for ICSI or IVF! The slides are not sterile, they contain endotoxins and may be embryotoxic. Sperm selection on HBA® slides is prohibited by the License.

Storage:
HBA® slides should be stored in a dry environment in its original packaging or in a clean, dry, dust-free environment at room temperature (20-28°C).

Performing the Assay:
Materials Provided:
- HBA® slides
- Cell-Vu® gridded cover slips
- Product Instructions for Use

Required Materials not Provided:
- Phase contrast microscope with at least 400x magnification
- Disposable, sterilized cover tests
- Mechanical counting device
- Human tubal fluid (HTF, Irvine Scientific, Santa Ana, CA) or other acceptable sperm diluent containing >0.50 mg/mL protein
- Disposable specimen handling gloves
- Timer/clock
- Pipette to deliver 2-10 µL, disposable pipette tips

Useful Accessories Not Provided:
- Microscope reticle delineating area
- Toluylene Blue O (Aldrich Chemical Co.)
- Urea (Aldrich Chemical Co.)

Step-by-step Procedure:
1. Perform the assay at 20-30°C. Do not exceed 30°C.
2. Land an assay chamber with the semen sample.
3. Immediately before use, mix the sample and the droplet of sperm diluent. The cell is prepared by estimating the proportion of mature sperm in the semen sample.
4. Immediately install the Cell-Vu® gridded cover slip: avoid entrapping air bubbles.
5. The cover slip provides a grid of 100 squares, 0.1 x 0.1 mm, within a viewing circle for 100 X magnification.

The coefficient of variation (CV) of the result is typically about 5%. Higher variances result when fewer sperm are counted.

Table 1

<table>
<thead>
<tr>
<th>Inter- and Intra-lot Precision with a Normal Specimen</th>
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<tbody>
<tr>
<td><strong>HBA® slide lot</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>3</td>
</tr>
</tbody>
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*on average, the sperm in nine grid squares were counted.

Rationale

Example 1:
A count of 115 bound, motile sperm was observed by counting 22 grid squares. Immediately after, a count of 9 unbound, motile sperm was observed from the same 22 grid squares.

Example 2:
In a different sample, a count of 89 unbound, motile sperm was observed by counting 100 grid squares. Immediately after, a count of only 27 bound, motile sperm was observed from all 100 grid squares.

Precision of the Assay:
The precision of the assay is related to the number of sperm counted. When the total count of bound and unbound sperm is between 100-200, the coefficient of variation (CV) of the result is typically around 5%. Higher variances result when fewer sperm are counted. Results are acceptable when at least 30 total motile sperm are counted.

In a study of variability within and among three production lots of slides, single semen specimens were assayed in replicates of assay slides, all within 3 hours of semen collection. Three different individuals stained the slides. Table 1 shows the results for a normal semen specimen; Table 2 shows results for an abnormal oligospermic specimen.

Table 1

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In the prediction of fertility, it is not necessary that all contribution to the zygote. Thus, hyaluronan-binding also associated with high genomic integrity (11, 14, with the oocyte complex and hyaluronan binding is sperm, in contrast, are competent in the interaction and therefore predicts infertility. Hyaluronan-binding low proportion of mature sperm in the semen sample. Defective cover slips occur rarely, clearly read. Otherwise, the label appears upside

e, and normal chromatin structure.

Table 2

| Inter- and Intra-lot Precision with an Abnormal, Oligospermic Specimen
<table>
<thead>
<tr>
<th>HBA® slide lot</th>
<th>Number of Replicates</th>
<th>Mean % Bound</th>
<th>Std. Dev.</th>
<th>Mean % Bound SD.</th>
<th>Methanol Bound</th>
<th>Mean % Bound - SD.</th>
<th>Methanol Unbound</th>
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<tbody>
<tr>
<td>A 20</td>
<td>59.0</td>
<td>10.9</td>
<td>18</td>
<td>21.5</td>
<td>14.8</td>
<td>15.6</td>
<td>11.3</td>
</tr>
<tr>
<td>H 10</td>
<td>55.8</td>
<td>19.0</td>
<td>34</td>
<td>20.1</td>
<td>16.0</td>
<td>17.6</td>
<td>12.6</td>
</tr>
<tr>
<td>12</td>
<td>57.1</td>
<td>13.1</td>
<td>23</td>
<td>21.3</td>
<td>16.6</td>
<td>17.8</td>
<td>13.2</td>
</tr>
</tbody>
</table>
| *The sperm in all 100 grid squares were counted.

Quality Control:

Quality semen samples can be used to demonstrate the sperm-hyaluronan binding reaction; however, fresh semen controls are not commercially available. Users are encouraged to establish their own sources of fresh control semen and to develop an associated history of normal and abnormal HBA® scores.

The sperm-hyaluronan binding reaction is not altered by freezing and thawing of sperm (16, 20, 25). Similarly, sperm can be either by swim up or by gradient preparation bind normally to hyaluronan.

The presence of the hyaluronan layer can be confirmed by flooding the assay chamber for a minute with a solution of toluidine blue O in 8 M urea then rinsing with water. A purple stain denotes the presence of hyaluronan (12). Do not use the stained chamber for assay; the dye inhibits sperm motility.

Expected Results:

The percent of hyaluronan-bound sperm ranges from essentially zero to essentially 100%. In a study of 157 semen samples processed for diagnosis of suspected infertility, the upper half of the samples showed an average HBA® score of 93 ± 26% binding. The lower half had HBA® scores from 88% to zero binding, the average was 70 ± 18% binding. Approximately 1% of these samples showed no detectable binding to hyaluronan.

HBA® scores on prepared sperm may differ from those of raw semen.

Limitations and Troubleshooting:

Use only fresh semen, less than three hours old.

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