CooperSurgical

HBA®

Sperm-Hyaluronan Binding Assay

CE
℞Only

PROPRIETARY AND ESTABLISHED NAMES:

HBA® Sperm-Hyaluronan Binding Assay

INTENDED USE:

The HBA® Sperm-Hyaluronan Binding Assay is an in vitro diagnostic indicated for use:

1. As a component of the standard analysis of semen in the diagnosis of suspected male infertility.
2. As a component of analyses of either raw or processed semen for determining the proper course of IVF treatment of infertility.

SUMMARY:

In natural fertilization mature 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® Binding Assay. Viewed in the microscope, bound sperm are differentiated from unbound sperm by their beating tails with heads that make no progressive movement.

Hyaluronan binding indicates the successful completion of spermiogenic events mediated by the HspA2 chaperone protein (1, 2, 8). In the absence of the chaperone, deficits in meiosis, DNA repair, hyaluronan binding and other steps in sperm maturation may occur.

Sperm unable to bind hyaluronan share many aspects of immaturity: they retain cytoplasm and histones (9), they show a higher frequency of aberrant morphology (18, 22, 23) and they have a lower genomic integrity than hyaluronan binders – hyaluronan binders have many fewer DNA single strand breaks than non-binders (24) and a 4-to-6 fold lower frequency of chromosomal aneuploidies (10, 11). Sperm motility is stimulated upon binding hyaluronan (6, 7, 14). Thus, the ability to bind hyaluronan designates a mature sperm and the proportion of mature sperm indicates the maturity of sperm in a semen sample.

THE HBA® SLIDE:

The HBA® slide has two identical hyaluronan-coated assay chambers. By counting the numbers of motile bound and unbound sperm in a common area, the proportion of binders can be calculated.

SPECIMEN COLLECTION AND PREPARATION:

Semen should be obtained by masturbation, preferably following 2-3 days of abstinence, and collected in a clean, dry container. Keep it at 20-28ºC for 30 minutes to liquefy. Samples still viscous after 30 minutes can be diluted in an equal volume of a sperm dilution medium. Perform the assay on the semen specimen within 3 hours of collection.

WARNINGS AND PRECAUTIONS:

1. *Semen is a hazardous fluid and must be handled and disposed of using normal precautions for infectious, biohazardous materials. Wear gloves, eye protection and a laboratory coat. Disinfect all waste and dispose of it in a 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. Purchase of this kit does not allow sperm selection on HBA® slides.*
3. *Unused slides may be discarded in regular municipal trash containers. However, slides that have been exposed to body fluids must be discarded as bio-hazardous waste. Broken slides should be discarded as sharps.*

STORAGE:

HBA® slides should be stored in a clean, dry environment at 20-28°C.

PERFORMING THE ASSAY:

Materials Provided:	Materials not Provided:
HBA® slides	Microscope with 400x magnification
Cover slips	Disposable, sterile capped test tubes
Product Instructions for Use	Mechanical counting device
	Human tubal fluid (HTF) 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
	Microscope reticle delineating area

STEP-BY-STEP PROCEDURE:

1. Perform the assay at 20-30° C.
2. Load one of the two assay chambers with semen.

   Immediately before use, mix the semen and pipette a drop, 7-10 µL in volume, onto an area near the outside edge of the assay chamber, making certain it stays within the chamber.
3. Immediately install the cover slip: avoid entrapping air bubbles.

   The cover slip provides a grid of 100 squares, 0.1 x 0.1 mm, within a viewing circle. Install the cover slip with the circle upward. Avoid air bubbles by contacting one edge of the cover slip to the edge of the chamber, forming an angle like two arms of a hinge (Figure 1); slowly lower the opposite edge of the cover slip, closing the hinge – when the cover slip is quite close to the slide, it will contact the sample drop (Figure 2); continue to lower the cover slip without releasing it; release the cover slip when the circle is completely covered with sample (Figure 3); finally center the circle over the chamber.

Figure 1

Contact cover slip to the slide:

Figure 2

Contact cover slip to the sample:

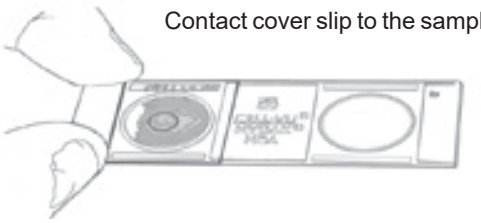

Figure 3

Cover slip in place:

4. Once the coverslip is placed, the incubation period begins. Do not delay placement of the coverslip. Uneven distribution of binding may occur, affecting results. Incubate the chamber for at least 10 and not more than 20 minutes. Count the unbound, motile sperm and the bound, motile sperm in the same number of grid squares (area).

   In 10 minutes all the sperm will contact and bind to the immobilized hyaluronan layer. After 20 minutes weak motile sperm may begin to lose motility.

   Bound motile sperm will cease progressive movement but retain active tail beating. Dead and non-motile sperm show no tail movement. Non-binding motile sperm swim about freely. Acceptable assay precision will be achieved when the sum of bound plus unbound motile sperm is greater than 200. With oligospermic samples, it may be necessary to count the sperm in several whole microscope fields, outside the grid area marked on the coverslip, in order to count 200 motile sperm.

   It will not always be possible to find 200 motile sperm in a semen sample, particularly in oligospermic samples

5. Calculate the percent hyaluronan-binding sperm.

   The percent of sperm binding to the hyaluronan layer is calculated as follows:

$$\%\ Bound = 100\ X\ \frac{Bound\ Motile\ Sperm}{Bound\ Motile\ Sperm + Unbound\ Motile\ Sperm}$$

   Example:

   A count of 89 unbound, motile sperm was obtained by counting 100 grid squares. Immediately after, 27 bound, motile sperm were counted in the 100 grid squares.

$$23\ \%\ Bound = 100\ X\ \frac{27\ Bound\ Motile\ Sperm}{27\ Bound\ Motile\ Sperm + 89\ Unbound\ Motile\ Sperm}$$

PRECISION OF THE ASSAY:

The precision of the assay is related to the number of sperm counted. The more sperm counted, the lower the variance. Preferably count at least 100 total motile sperm; 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. With a normal specimen, counting approximately 196 sperm per replicate, the results (means (% bound)/CVs (%)/ and numbers of replicates) were: (86.9, 85.5, 85.0/4, 5, 4/20, 10, 10). With an abnormal, oligospermic specimen, counting approximately 41 sperm, the results were: (59.0, 55.8, 58.4/18, 34, 17/20, 10, 10).

QUALITY CONTROL:

Fresh mature semen 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, 26). Similarly, sperm processed either by swim up or by gradient preparation bind normally to hyaluronan.

EXPECTED RESULTS:

The percent of hyaluronan-bound sperm ranges from essentially zero to essentially 100%. In a study of 157 semen samples from patients presenting for diagnosis of suspected infertility, the upper half of the samples showed an average HBA® score of 93 ± 2.6% 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.

Sperm preparation (swim-up, gradient) can alter the proportion of HA binders in a semen sample.

LIMITATIONS AND TROUBLESHOOTING:

Use only fresh semen, less than three hours old.

The sample should permit at least 30 motile sperm to be counted. If less than 30 sperm occur in the grid, install a microscope reticle or count successive different microscope fields to permit inspection of an area large enough to evaluate at least 30 motile (bound plus unbound) sperm. Alternatively, concentrate the sample and re-assay the concentrate.

Always be sure that the sample has been applied to the coated side of the slide. The coated side is up when “HYDAK® HBA” can be clearly read. Application of sperm and cover slip to the uncoated side will give the appearance of no sperm binding or, if the cover slip has been pressed down hard, the sperm may appear immotile.

Whenever no binding is observed, repeat the assay of the sample on a different slide to confirm the result.

Occasionally defective cover slips occur with the grid etched on top of the slip instead of on the side in contact with the sample. When this occurs, repeat the assay with a new cover slip. Contact your distributor for replacements.

If an air bubble blocks counting within the counting grid, repeat the assay with a new chamber and cover.

SIGNIFICANCE OF THE RESULTS:

A low HBA® score reflects a low proportion of mature sperm in the semen and therefore predicts infertility. Hyaluronan-binding sperm, in contrast, are competent in the interaction with the oocyte and are associated with high genomic integrity (11, 14, 21, 25), which improves the quality of the paternal contribution to the zygote. Hyaluronan-binding differentiates high and low functional integrity and fertilizing potential.

In prediction of fertility, it is not necessary that all sperm should be capable of binding hyaluronan, but the proportion of hyaluronan binders must reach an effective level in order to achieve a good likelihood of fertility. Binding above the effective level probably does not further improve fertility. Based on correlations with normal sperm morphology, the Hyaluronan Binding Assay level differentiating higher from lower expectation of fertility is estimated to be approximately 80%, Table 1

Table 1

HBA® Score Results Interpretation	
HBA® Score (% binding)	**Interpretation**
≥80 % binding	Normal maturity and physiological function
<80 % binding	Diminished maturity and physiological function

PERFORMANCE CHARACTERISTICS:

A study comparing HBA® score results to normal sperm morphology (strict criteria) was carried out with semen samples from three IVF or andrology laboratories in three states (CT, CA and PA), with patients presenting for diagnosis of suspected infertility. At least 50 samples were obtained from each site and the HBA® assay performed on site according to the HBA® Instructions for Use. Morphology slides were prepared on site and sent to a central location where they were scored by a single reader.

The data were analyzed by a two-way contingency table using morphology (classified as subfertile if <5% normal morphology, otherwise fertile (4, 5, 15, 17)) and HBA® score (classified as subfertile if <80% binding, otherwise fertile). Significance was tested with the Pearson Chi-squared statistic using SPSS for Windows software. The analysis showed that HBA® score was significantly related to morphology for data from the combined data (all three sites), Table 2.

The combined data showed good specificity and positive predictive values, although sensitivity was only 40%. Therefore, at a cutoff of <80% binding, the HBA® assay detects less than half of semen samples with truly low morphology, but among those detected as low HBA® score there is a strong prediction (>80%) of poor morphology.

In a subsequent clinical trial with human outcomes, an HBA® Score of 65% was used as the key cutoff (24).

Table 2

Significance of HBA® Score to Morphology					
Study Site	**N**	**Sensitivity**	**Specificity**	**Positive Predictive Value**	**p =**
PA	50	43.2	76.9	84.2	0.198 (NS)
PA-r◊	50	51.3	90.9	95.2	0.012
CA	52	54.2	78.6	68.4	0.015
CT	55	26.5	100	100	0.01
Combined	157	40.0	85.5	80.9	0.001

◊Site data recalculated with cutoffs of <5% for morphology and <80% for HBA®.

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SYMBOLS DEFINITION

Ref #	Symbol	Title	Description	Standard Development Organization
5.1.6		Catalogue number	Indicates the manufacturer's catalogue number so that the medical device can be identified.	ISO 15223-1
5.1.5		Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.	ISO 15223-1
5.4.3		Consult instructions for use	Indicates the need for the user to consult the instructions for use.	ISO 15223-1
5.3.4		Keep dry	Indicates a medical device that needs to be protected from moisture.	ISO 15223-1
5.2.7		Non-sterile	Indicates a medical device that has not been subjected to a sterilization process.	ISO 15223-1
5.4.2		Do not re-use	Indicates a medical device that is intended for one use, or use on a single patient during a single procedure.	ISO 15223-1
5.5.1		In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.	ISO 15223-1
5.2.8		Do not use if package is damaged	Indicates a medical device that should not be used if the package has been broken or damaged.	ISO 15223-1
5.3.7		Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed.	ISO 15223-1
5.1.4		Use-by Date	Indicates the date after which the medical device is not to be used.	ISO 15223-1
5.1.2		Authorized representative in the European Community.	Indicates the authorized representative in the European Community	ISO 15223-1
5.1.1		Manufacturer	Indicates the medical device manufacturer, as defined in EU Directive 90/385/EEC, 93/42/EEC and 98/79/EC.	ISO 15223-1
5.5.5		Contains sufficient for <n> tests	Indicates the total number of IVD tests that can be performed with the IVD.	ISO 15223-1
n.a.	R_xOnly	Prescription device	Caution: Federal law (USA) restricts this device to sale by or on the order of a licensed healthcare practitioner.	21 CFR 801.109
n.a		Product conforms to the Medical Device Directive 93/42/EEC	Signifies European technical conformity.	n.a.

Made in Costa Rica

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