

Axis-Shield Clinical Chemistry Heparin Binding Protein Reagent Kit



FHHBP101



Reag 1, 1 x 21.0 mL
Reag 2, 1 x 5.0 mL
100 Tests



ENGLISH

FOR PROFESSIONAL USE ONLY

INTENDED USE

The Axis-Shield Heparin Binding Protein (HBP) test is an immunoturbidimetric assay for the quantitative determination of Heparin Binding Protein in human plasma.

The Axis-Shield Heparin Binding Protein assay is intended for use in conjunction with other laboratory findings and clinical assessments as an aid in the assessment for the presence of organ dysfunction, or risk of progression to organ dysfunction within 72 hours, in patients presenting to the Emergency Department (ED) with suspected infection.

SUMMARY

Heparin Binding Protein (HBP), also known as Cationic Antimicrobial Protein of 37kDa (CAP37) and azurocidin, is a 37kDa glycoprotein synthesised in neutrophils.¹

Structurally HBP belongs to the serine protease superfamily and although it has 45% sequence identity with human neutrophil elastase, it is inactive as a protease. Although initially investigated for its antimicrobial activity, HBP has since been implicated in a number of inflammatory processes.

HBP is released from the secretory vesicles of activated neutrophils on contact with the endothelium. Once released, it induces a calcium-dependent rearrangement of the endothelial cell cytoskeleton, resulting in cell contraction and increased permeability of the endothelium. It is also internalised by the endothelial cells to protect them from apoptosis. HBP is also released from the secretory vesicles when M-protein/fibrinogen complexes, which are formed when M-proteins are released from bacterial cell surfaces, interact with I2-integrins on the neutrophil cell surface.

At the site of infection, HBP is also secreted from the azurophil granules during phagocytosis, where it exhibits antimicrobial activity and is responsible for the recruitment and activation of monocytes and other inflammatory mediators. It is also internalised by monocytes to prolong survival and enhance cytokine production.²

HBP therefore directly contributes to the maintenance and progression of inflammation and it has been proposed that measurement of HBP could be useful in identifying patients at risk of developing sepsis with circulatory failure.

A publication in 2015 demonstrated that in febrile patients presenting to the Emergency Department (ED), HBP is an early indicator of infection-related organ dysfunction, and a strong predictor of disease progression to severe sepsis within 72 hours.³ In this prospective study, 806 febrile adult patients with suspected infection were enrolled, of those, 487 were confirmed as having an infection but no organ dysfunction at presentation. In this 487 cohort, 141 progressed to develop organ dysfunction within 72 hours and in this sub-group HBP showed a sensitivity for prediction of organ dysfunction of 78.0%, a specificity of 76.3%, a positive predictive value (PPV) of 57.3% and a negative predictive value (NPV) of 89.5% which exceeded those values obtained for all other markers tested (Procalcitonin, White Cell Count, C-reactive Protein, Lactate). Receiver-operating characteristic (ROC) curves also demonstrated that HBP was the best predictor of infection-related organ dysfunction (severe sepsis), with an area under the curve (AUC) value of 0.80 (Confidence Interval (CI) 95% 0.76-0.85).

PRINCIPLE

The determination of HBP is based on a turbidimetric reaction which occurs between circulating Heparin Binding Protein and avian HBP monospecific polyclonal antibodies bound to chloromethyl microparticles at optimal pH conditions in the presence of polyethylene glycol polymer (PEG). The magnitude of the change is proportional to the quantity of HBP in the test sample.

KIT COMPONENTS

REAG 1	1 x 21.0 mL	PEG buffer, bovine serum albumin 0.05% sodium azide < 0.1% and detergent. READY TO USE
REAG 2	1 x 5.0 mL	Phosphate buffer, Ovalbumin 0.1%, avian anti-HBP antibody 0.03% coated on microparticles surface, sodium azide < 0.1% and detergents. READY TO USE

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- Clinical Chemistry system capable of measuring up to 600nm with 37°C incubation. Refer to the specific Axis-Shield Clinical Chemistry HBP Application Guide.
- Axis-Shield Clinical Chemistry HBP Control Kit, Product Code FHHBP200
- Axis-Shield Clinical Chemistry HBP Calibrator Kit, Product Code FHHBP300

WARNINGS AND PRECAUTIONS

- IVD** For *in vitro* diagnostic use.
- Disposal of all waste material must be in accordance with local guidelines.
- Reagents contain sodium azide which can react with lead and copper plumbing to form highly explosive metal azides. On disposal, drain with large quantities of water to prevent azide build-up.
- Material safety data sheets (MSDS/SDS) are available upon request from Axis-Shield.
- Caution: Federal law restricts this device to sale by or on the order of a physician.

 WARNING REAG 1	WARNING H317 –	May cause an allergic skin reaction
	PREVENTION P261 – P272 – P273 – P280 – P302+352 – P333+313 – P363 –	Avoid breathing dust/fumes/gas/mist/vapours/ spray. Contaminated work clothing should not be allowed out the workplace. Avoid release to the environment. Wear protective gloves/protective clothing/eye protection/face protection. IF ON SKIN: Wash with plenty of water. If skin irritation or rash occurs. Get Medical attention. Wash contaminated clothing before use.

REAGENT STORAGE AND STABILITY

Opened (in-Use) Kit Stability

- Reag 1 and Reag 2 are stable at 2-8°C for up to:
 - Opened – 15 days
- For on-board storage capabilities refer to the specific Axis-Shield Clinical Chemistry HBP Application Guide.

Unopened kit stability

All components are stable until expiration as directed on the label at 2-8°C. Reagents must not be frozen.

Handling and Procedural Notes

- Store kit components at 2-8°C.
- Reagents are supplied ready for use, but for some instruments, these may require transfer to appropriate reagent holders. Refer to the specific Axis-Shield Clinical Chemistry HBP Application Guide.
- ENSURE HOMOGENEITY OF REAGENT 2 BEFORE USE BY GENTLE INVERSION OF THE VIAL.**
- Do not use beyond the expiration date.
- DO NOT FREEZE REAGENTS.**
- Reagents must be returned to 2-8°C storage after use if they cannot be stored on-board under refrigerated conditions in the original packaging.
- Do not mix reagents between different lot numbers.
- Use a new disposable pipette tip for each reagent or sample manipulation to avoid contamination.
- Un-mixed reagents should be clear of particulate material and should be discarded if they become turbid.

Indications of Deterioration

Values outside the recommended acceptable range for the Axis-Shield Clinical Chemistry HBP controls may be an indication of reagent instability and associated results are invalid. The kit should be discarded and the samples retested.

SAMPLE STORAGE, COLLECTION AND HANDLING

Collection and Handling

- For sample collection and preparation, only use suitable tubes or collection containers.
- Only the sample matrix listed is suitable for use:
 - Plasma (sodium citrate).
- Other sample collection tubes have not been verified for use. For optimum performance Becton Dickinson 0.105 Molar / 3.2% Sodium citrate tubes (4.5 mL) are recommended.
- Do not use grossly haemolysed or turbid samples.
- Thoroughly mix thawed samples before assay and avoid repeated freeze/thawing.
- Do not use heat-inactivated samples, this may yield incorrect results.
- Use caution when handling patient samples in order to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each sample to prevent cross contamination.⁴
- HBP is stored in and released from neutrophils;⁵⁻⁷ handling of the separated plasma in relation to the packed cells is therefore critical. Care should be taken to avoid the buffy coat and cell layer when aspirating from the draw tube to avoid the risk of contaminating the plasma with neutrophils from the cell layer.

Preparation for analysis

- When processing samples follow the instructions provided by the collection tube manufacturer.
- All human samples should be considered potentially infectious. It is recommended that these materials be handled in accordance with local/national guidelines on laboratory safety procedures.
- Fresh/Non-Frozen Samples:
 - Samples must be mixed thoroughly prior to use.
- Frozen Samples:
 - Thaw samples thoroughly for a minimum of 30 minutes.
 - Mix thawed samples thoroughly by inverting a minimum of 5 times.

Sample Storage and Stability

This procedure can be performed with human citrated plasma specimens. Grossly lipaemic, haemolysed or microbial contaminated samples may give poor results and should not be used.

Sample stability can be supported at the following storage conditions:

Storage	Claim
Room Temperature (20°C)	Up to 4 days
2-8°C	Up to 4 days
On-board	Up to 3 hours
Freeze/thaw cycles	Up to 1 cycle
Frozen ($\leq -70^{\circ}\text{C}$)	Up to 26 months

For longer term storage, samples MUST be stored frozen at -70°C or colder.

Dispose of any used materials in accordance with local waste management regulations.

ASSAY PROCEDURE

- Program the instrument using the appropriate instrument-specific protocol. Refer to the specific Axis-Shield Clinical Chemistry HBP Application Guide.
- Invert reagents to facilitate mixing and then load reagents and samples as per specific Axis-Shield Clinical Chemistry HBP Application Guide.

STANDARDIZATION

Currently there is no international reference standard for this assay. The Axis-Shield Clinical Chemistry HBP assay is traceable to internal reference calibrators.

CALIBRATION

For assay calibration use the Axis-Shield Clinical Chemistry HBP Calibrator materials as listed in the "Equipment and Materials Required but not Provided" section.

Refer to the calibrator vial labels for specific concentrations.

Calibration Frequency:

Calibration curve stability is instrument specific. Refer to the specific Axis-Shield Clinical Chemistry HBP Application Guide.

Recalibration is also recommended after a change in reagent lot, if a control reads out-of-range or as required following quality control procedures.

QUALITY CONTROL

For quality control, use Axis-Shield Clinical Chemistry HBP Control materials as listed in the "Equipment and Materials required but not provided" section. Maintenance and calibration of the instrument must be performed according to the operator's instruction manual for the specific analyser.

Users should ensure they understand the instructions of this assay, particularly the Warnings and Precautions, Sample Handling and Procedure Limitations sections. It is recommended that Axis-Shield Clinical Chemistry HBP Controls and Calibrators are run in duplicate each day of use.

The control limits should be established by individual laboratories and in accordance with laboratories quality control procedures and/or any local or government guidelines.

RESULTS

Unit of Measure

The default unit for the assay is ng/mL.

Dilutions

Automatic on-board sample dilution 1:5 (1 part sample + 4 parts 0.9% saline) has been verified for samples up to 600 ng/mL. Sample recovery was within 90 – 110% of expected HBP concentrations.

Samples above the measuring range can be manually diluted 1:5 (1 part sample + 4 parts 0.9% saline).

Interpretation of Results

- For interpretation of results, refer to the specific Axis-Shield Clinical Chemistry HBP Application Guide for suitable curve fit algorithms.

EXPECTED VALUES

A Reference Range study was performed according to CLSI document EP28-A3C.⁸ The study was performed using human citrated plasma samples. This comprised of 19 males (age 21 – 54 years), 28 females (age 20 – 66 years) and 6 normal samples with unknown demographic details. Samples were analysed using three reagent lots. The following representative results are tabulated below:

95% Reference limit ng/mL (90 % CI)	Overall Mean (ng/mL)	Overall Median (ng/mL)
21.44 (19.34 – 23.54)	9.40	10.92

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range (s). Consider these values as guidelines only.

ANALYTICAL PERFORMANCE CHARACTERISTICS

Representative data from testing on the Siemens ADVIA 1800 analyser is presented; results from individual laboratories may vary. Other systems are not supported unless an instrument application guide is provided.

PROZONE

Prozone effect is whereby specimens with high levels of analyte may assay within the measuring interval of the assay. HBP levels up to 1080 ng/mL did not display any prozone effect.

LIMIT OF DETECTION

The limit of detection (LOD) of the assay was determined to be 8.01 ng/mL.

The LOD is defined as the lowest concentration of analyte that can be detected with greater than or equal to 95% probability as described in CLSI Document EP17-A2.⁹

SAMPLE CARRYOVER

Within assay sample carryover was assessed. No carryover was observed as defined by $\leq 10\%$ when a low HBP sample was preceded by a high sample.

ASSAY PRECISION

A precision study was performed according to CLSI Document EP5-A3.¹⁰ Five patient samples were analysed using three lots of reagents. Each sample was tested in replicates of two at two separate times per day for 20 days (n=80). Data from one lot tested is summarised below as representative data.

Sample	n	Mean HBP ng/mL	Total (Within Lab)		Repeatability (Within Run)	
			SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
Sample 1	80	18.9	1.8	6	1.3	4
Sample 2	80	42.4	2.7	5	1.2	2
Sample 3	80	81.6	5.1	5	1.7	2
Sample 4	80	118.4	8.2	5	1.8	1
Sample 5	80	213.0	21.0	7	3.5	1

ANALYTICAL MEASURING RANGE/LINEARITY

Linearity was assessed as per CLSI Document EP6-A.¹¹ The assay demonstrated linearity from 8.4 – 337.0 ng/mL.

METHOD COMPARISON

A method comparison study was performed according to CLSI document EP9-A3.¹² A correlation study was performed using human citrated plasma samples. Samples were analysed using three Axis-Shield Clinical Chemistry HBP reagents lots and one commercially available HBP EIA assay. The samples were evaluated using Passing-Bablok regression methods and the correlation was assessed by Pearson (r) correlation method. The following results were obtained:

Lot	Sample Range EIA (ng/mL)	n	Correlation coefficient (r)	Slope (95 % CI)	Intercept (95 % CI)
1	6.60 – 193.30	105	0.96	0.91 (0.84 – 0.97)	-1.31 (-5.41 – 2.34)
2	6.60 – 193.30	113	0.96	0.93 (0.87 – 0.99)	2.17 (-0.21 – 4.65)
3	6.60 – 193.30	114	0.97	1.00 (0.93 – 1.05)	0.77 (-1.19 – 3.66)

INTERFERENCES

Interfering substances were tested as per CLSI Document EP07-A2.¹³ The levels of substances tested as indicated below did not interfere in the assay as defined by an observed bias (%) of less than 10%

Interfering Substances	Test Concentration
Bilirubin (Conjugated)	Up to 9 mg/dL
Bilirubin (Unconjugated)	Up to 21 mg/dL
Haemoglobin	Up to 1674 mg/dL
Triglyceride	Up to 850 mg/dL
Total Protein	Up to 12 g/dL

Interference from administered drugs was assessed as per CLSI Document EP07-A2.¹³ The levels of substances tested as indicated below did not interfere in the assay as defined by a change in HBP concentration of less than 10%:

Interfering Substances	Test Concentration
Cefotaxime	0.9 mg/mL
Dopamine	0.15 mg/mL
Dobutamine	12.24 $\mu\text{g}/\text{mL}$
Furosemide	0.022 mg/mL
Heparin	5 IU/mL (5000 IU/L)
Imipenem	1.25 mg/mL
Noradrenaline	2.3 $\mu\text{g}/\text{mL}$
Penicillin G	0.45 mg/mL
Vancomycin	3.85 mg/mL

CROSS-REACTIVITY

Cross-reactivity was assessed at two HBP concentrations (40 ng/mL and 300 ng/mL) in the presence 1 µg/mL Cathepsin G, Human Neutrophil Elastase or Proteinase 3. No cross-reactivity was observed as defined by a change in HBP concentration of $\leq 10\%$.

LIMITATIONS OF USE

- No reagent carryover studies have been performed for this assay.
- Clinical decisions should not be based solely on the Axis-Shield HBP assay results. Interpret test results in conjunction with the patient's history, clinical status, physical findings and other diagnostic procedures.
- Treatment should not be initiated on the sole basis of an elevated HBP test result nor treatment withheld on the sole basis of a low result.
- HBP is stored in and released from neutrophils and as such results from neutropenic and neutrophilic patients should be interpreted with caution. Axis-Shield has not conducted any evaluations to assess assay performance in all potential neutropenic or neutrophilic populations. Neutropenic populations may include but are not limited to cancer patients, chemotherapy-induced or drug-induced neutropenia and various autoimmune conditions. Neutrophilic populations may include but are not limited to malignancy, myeloproliferative and leukemic disorders, tissue damage including infarction and various drugs, particularly corticosteroids.
- HBP may also be elevated (unrelated to sepsis risk) in conditions of systemic inflammation including but not limited to severe burns, whole body ischaemia and major trauma including surgery.
- The use of this device as a companion diagnostic has not been established.

CLINICAL PERFORMANCE DATA

Representative data established using the Axis-Shield Heparin Binding Protein EIA; results in individual laboratories may vary.

Results reported are from a prospective observational study performed at multiple sites in Sweden and the USA.

In a representative study of 487 adult (>18 years) patients presenting to the Emergency Department with suspected infection and one or more signs of a systemic response but no evidence of organ dysfunction, the following clinical performance was observed:

HBP	Development of Infection-related Organ Dysfunction within 72 hours of ED presentation		
	Positive	Negative	Total
Positive (>30 ng/ml)	108	83	191
Negative (<30 ng/mL)	33	263	296
Total	141	346	487

Statistic	Performance (95% CI)
Clinical Sensitivity	76.6% (68.7 – 83.3%)
Clinical Specificity	76.0% (71.2 – 80.4%)
Positive Predictive Value (PPV)	56.5% (51.4 – 61.6%)
Negative Predictive Value (NPV)	88.9% (85.5 – 91.5%)

From this representative study data, the following cut-off is suggested for the risk assessment of ED patients presenting with suspected or confirmed infection:

Sample Result (ng/mL)	Risk of development of infection-related organ dysfunction within 72 hours
≤ 30	Lower risk
> 30	Higher risk

The clinical findings, signs, symptoms, physician's impression and other laboratory results must also be considered when interpreting the results of this assay.





TECHNICAL ASSISTANCE

For any technical assistance contact your local distributor or Axis-Shield Diagnostics Limited.

e-mail: AXD.productsupport@alere.com

BIBLIOGRAPHY

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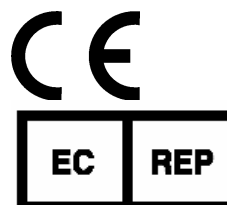
IVD	In Vitro Diagnostic Medical Device
REF	Product Code
LOT	Lot Number
Σ	Number of tests
	Consult Instructions For Use
	Manufacturer
	Use by
	Store at 2-8 °C
REAG 1	Reagent 1
REAG 2	Reagent 2
Rx Only	For Prescription Use Only

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