

DESIGN VERIFICATION

BLUESTAR®

Title: Verification Report for HEXAGON OBTI (Test for confirming the presence of human blood traces)

Version No.: 002

Valid from: 30.01.2006

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1 Function

HEXAGON OBTI has been designed as a rapid test for confirming the presence of human blood traces. The test is based on an immunochromatographic technique, featuring immobilized monoclonal antibodies and a coloured particular reagent. Blood samples are taken with a dedicated device which allows a clean and secure sampling. In the sample transportation tube hemoglobin is lysed from erythrocytes and preserved for at least one week at room temperature. The test is performed by breaking off the tip of the sample transportation device and dispensing two full drops onto the sample window of the test device. When the solution migrates along the test device two blue lines will appear if hemoglobin is present in concentrations $> 0.05 \mu\text{g/ml}$. A single blue line on the far end of the result window is an indication for a negative result.

Due to the use of monoclonal antibodies the test is highly specific for human hemoglobin and is not affected by animal proteins, vitamins, drugs etc.

HEXAGON OBTI is supplied in a convenient package, consisting of individually blistered test devices and sample transportation tubes.

2 Sensitivity and dynamic range

2.1 Description of control materials

Purified native human hemoglobin is employed (Sigma Diagnostics, or ProDiamed). A stock solution is prepared by dissolving 10 mg of human hemoglobin in 1 ml phosphate-buffered saline (PBS). From this stock solution working standards are prepared by diluting appropriate volumes in sample transport medium according to the following scheme:

Origin	μl	Diluent, μl	Final concentration, $\mu\text{g/ml}$	Cal. #
Stock	200	800	2000.00	10
Stock	100	900	1000.00	9
Stock	50	950	500.00	8
Stock	20	980	200.00	7
Stock	10	990	100.00	6
Cal. #6	100	900	10.00	5
Cal. #6	50	950	5.00	4
Cal. #6	10	990	1.00	3
Cal. #3	100	900	0.10	2
Cal. #3	50	950	0.05	1
---		1000	0.00	0

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2.2 Results

All concentrations are tested in double. From each standard solution 100 µl are applied to the test device (equal to two full drops from the sample transportation tube). The results are interpreted after 5 and 10 minutes.

	Cal.#0	Cal.#1	Cal.#2	Cal.#3	Cal.#4	Cal.#5	Cal.#6	Cal.#7	Cal.#8	Cal.#9	Cal.#10
Hb, µg/ml	0	0.05	0.1	1	5	10	100	200	500	1000	2000
TL, 5'	-	-	±	2+	3+	3+	4+	4+	4+	3+	+
CL, 5'	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
TL, 10'	-	±	+	3+	4+	4+	4+	4+	4+	3+	2+
CL, 10'	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+

Interpretation for test lines (TL) and control lines (CL) at 5 and 10 minutes:

(-) no line; (±) weak line; (+) visible line; (2+) medium intense line; (3+) strong intense line; (4+) very strong intense line

From the above the test shows a sensitivity of 0.05 µg/ml hemoglobin and a dynamic measuring range of 0.1 - 2000 µg/ml.

3 Specificity, cross-reactivity and interferences

3.1 Description of control materials

Purified human hemoglobins HbA₀, HbA₂ and HbS were purchased from Sigma Diagnostics. Purified human HbF was purchased from New England Immunology Corp. Purified animal hemoglobins (bovine, horse, pig, sheep, goat, rabbit and turkey) were purchased from Sigma Diagnostics. The proteins human albumin, human IgG, human transferrin and horse radish peroxidase (HRP) were also purchased from Sigma Diagnostics.

3.2 Results

Purified native human hemoglobin as well as HbA₀, HbA₂, HbF and HbS were employed in a concentration of 1 µg/ml. The test yielded positive results with HbA₀, HbA₂ and HbF, the relative intensities of the test lines varied between 2+ and 3+. With HbS the test line was slightly weaker (1+ to 2+).

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Results from the cross-reactivity studies are summarized in the following table.

Substance	Upper limit, µg/ml	Test line, rel. intensity (time)	Control line, rel. intensity (time)
Bovine hemoglobin	1000	- (10 min)	4+ (10 min)
Sheep hemoglobin	1000	- (10 min)	4+ (10 min)
Goat hemoglobin	1000	- (10 min)	4+ (10 min)
Horse hemoglobin	1000	- (10 min)	4+ (10 min)
Rabbit hemoglobin	1000	- (10 min)	4+ (10 min)
Turkey hemoglobin	1000	- (10 min)	4+ (10 min)
Pig hemoglobin	1000	- (10 min)	4+ (10 min)
Human albumin	1000	-/(±) (10 min)	4+ (10 min)
Human IgG	1000	- (10 min)	4+ (10 min)
HRP	1000	- (10 min)	4+ (10 min)

In conclusion, HEXAGON OBTI is highly specific for human hemoglobin and showed virtually no cross-reactivity with animal hemoglobins at concentrations up to 1000 µg/ml. Also no cross-reactivity could be observed with major human plasma proteins and HRP with the possible exception of albumin, where a faint test line appeared at 1000 µg/ml in a single case.

3.3 Interferences

A number of potentially interfering substances have been added to sample transport medium with and without human hemoglobin present. Bilirubin up to 30 mg/dl, triglycerides (employed as Intralipid standard material) up to 1000 mg/dl, paracetamol up to 20 mg/dl, acetylsalicylic acid (Aspirin) up to 20 mg/dl and ascorbic acid (Vitamin C) up to 100 mg/dl did not affect the negative and positive results, respectively.

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4 Clinical evaluation

4.1 Study Krankenhaus Nordwest, Frankfurt Germany

HEXAGON OBTI has been evaluated in the Krankenhaus Nordwest, Frankfurt, Germany. A total of 96 stool samples have been tested with both HEXAGON OBTI and a routine guajac method. The results are summarized below.

No.	Sample	Hex. OBTI	Guajac	Clin. diagnosis
80	normal	80 negative	80 negative	all negative
3	Iron suppl.	3 negative	3 positive	all negative
4	normal, hemoglobin added, Vit.C uptake	4 strong positive	3 negative, 1 weak positive	hemoglobin added
3	<i>Clostridium diff.</i> infection	3 strong positive	2 doubtful, 1 weak positive	all positive
4	unspec. collitis	4 strong positive	4 positive	all positive
2	colorect. carcinoma	2 strong positive	2 positive	all positive

From the above it is evident that HEXAGON OBTI gave no indication for false positive results. All samples containing hemoglobin, either from occult bleedings or spiked, were correctly found positive.

In conclusion, HEXAGON OBTI yielded a diagnostic sensitivity and specificity of 100%, respectively.

Total	Correct positives	Correct negatives	Diagn. sensitivity	Diagn. specificity
96	13	83	100%	100%

4.2 Study Klinik und Poliklinik für Kinderheilkunde, Münster, Germany

In another independent study a total of 30 young and adolescent patients (2 - 16 years old) under antirheumatic therapy have been monitored with HEXAGON OBTI. The patients have been treated with the following drugs: MTX, Prednisolon, Azathioprin, Naproxen, Diclofenac, Sulfasalazin, Cloprednol, Indometacin, Hydroxychloroquin, Cyclosporin A and Cloprednol. All stool samples have been confirmed negative for hemoglobin by two independent diagnostic tests and clinical investigations. HEXAGON OBTI showed negative results in all cases, thus excluding interferences by the above mentioned drugs.

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5 Stability

5.1 Real-time stability

The stability of HEXAGON OBTI has been demonstrated on real-time stability and temperature stress studies. Function tests of the finalized kit have been performed with the control materials described above.

Lot: H1049

Date of manufacture: 04/1997

Expiry: 01.07.1998 (18 months)

	Test line intensities compared on fresh and after 32 months							
µg/ml Hb	0	0.05	0.1	1	10	100	1000	2000
Fresh	-	(+)	2+	4+	4+	3+	1+	(+)
on 20.12.1999	-	(+)	2+	3+	3+	3+	(+)	((+))

Lot: H1049

Date of manufacture: 04/1997

Expiry: 01.07.1998 (18 months)

	Test line intensities compared on fresh and after 25 months							
µg/ml Hb	0	0.05	0.1	1	10	100	1000	2000
Fresh	-	(+)	2+	4+	4+	3+	1+	(+)
on 25.05.1999	-	(+)	2+	3+	3+	3+	(+)	((+))

Lot: H1039

Date of manufacture: 01/1997

Expiry: 01.04.1998 (18 months)

	Test line intensities compared on fresh and after 28 months							
µg/ml Hb	0	0.05	0.1	1	10	100	1000	2000
Fresh	-	(+)	2+	4+	4+	3+	1+	(+)
on 25.05.1999	-	((+))	1+	3+	3+	3+	(+)	((+))

Lot: H1029

Date of manufacture: 01/1997

Expiry: 01.04.1998 (18 months)

	Test line intensities compared on fresh and after 25 months							
µg/ml Hb	0	0.05	0.1	1	10	100	1000	2000
Fresh	-	(+)	2+	4+	4+	3+	1+	(+)
on 25.05.1999	-	((+))	1+	2+	3+	3+	(+)	((+))

The results of the continued real-time stability study together with the results from additional temperature stress tests (not displayed here) confirm a stability of more than 35 months. The results therefore support the stability claim of 21 months from the date of production.