Design Verification

HEXAGON OBTI

CONTENTS

1 Function

HEXAGON OBTI has been designed as a rapid immunological test for the qualitative detection of human hemoglobin in stool samples (faecal occult blood). Additionally, the test is suited for forensic purposes to determine whether blood traces are of human/primate origin.
HEXAGON OBTI is based on an immunochromatographic technique, featuring immobilised monoclonal antibodies and a coloured particular reagent. Stool samples are taken with a dedicated device which allows a clean and secure sampling. In the sample collection tube haemoglobin is lysed from erythrocytes and preserved for one week at room temperature or in a refrigerator. The test is performed by breaking off the tip of the sample transportation tube and dispensing three full drops onto the sample window of the test device. When the solution migrates along the test device two red lines will appear if hemoglobin is present in concentrations ≥ 0.05 µg/ml. A single red line on the far end of the result window is an indication for a negative result.
HEXAGON OBTI consists of individually sealed test cassettes and sample collection tubes.

2 Analytical sensitivity

2.1 Description of control materials

Purified native human haemoglobin (consisting of 98:2 HbA_1/HbA_2), human cord blood as source for haemoglobin F (HbF) and human sickle cell haemoglobin (HbS, Sigma Aldrich) are employed. Stock solutions are prepared by dissolving the lyophilized human haemoglobin preparations in TRIS/BSA buffer. From this stock solution working standards are prepared by diluting appropriate volumes in sample transport medium. The cord blood was directly diluted in the sample transport medium.

2.2 Results with human haemoglobin

	Concentration	Test result
Haemoglobin A	0	-
	30 ng/ml	+
	50 ng/ml	+/++
	100 ng/ml	+++
	1 µg/ml	++++
	100 µg/ml	++++
	2,000 µg/ml	++/+++
Haemoglobin S	30 ng/ml	-
	50 ng/ml	-
	100 ng/ml	-
	1 µg/ml	++
	100 µg/ml	++++
	2,000 µg/ml	++++
Cord blood	Approx. 48 ng/ml	++
	Approx. 95 ng/ml	+++
	Approx. 190 ng/ml	+++
	Approx. 1900 ng/ml	++++

Intensity of the test line:
- = negative; ± = very weak positive; + = weak positive; ++= medium intense line; +++ = strong positive; ++++ = very strong pos.

Conclusion: HEXAGON OBTI shows a sensitivity of at least 30 ng/ml with haemoglobin A and a dynamic measuring range of 0.03 – 2,000 µg/ml. Sickle cell haemoglobin reacts positive, however, the signal is less intense compared to haemoglobin A. Cord blood (that has a higher proportion of haemoglobin F) reacts positive with a reactivity similar to HbA. The test is resistant to the Hook effect up to 10000 µg/ml, where the signal can still be clearly seen.

3 Specificity, cross-reactivity and interferences

To evaluate cross-reactivity with haemoglobin derived from other species blood from various animals was tested. The results of these studies are summarised in the following table.

3.1 Results with animal blood

Haemoglobin obtained from	Approx. concentration in µg/ml	Test result
Cattle	1000	-
Pig	1000	-
Sheep	1650	-
Goat	500	-
Horse	100	-
Chicken	1000	-
Duck	1000	-
Goose	1000	-
Turkey	1000	-
Guinea pig	1000	-
Red deer	1000	-
Cat	1000	-
Dog	1000	-
Mouse	1000	-
Rabbit	2000	-
Badger	200	±
Weasel	500	+
Gorilla	500	+
Langur (Javan Lutung)	500	+

- = negative; ± = very weak positive ; + = positive

In conclusion, HEXAGON OBTI showed no cross-reactivity with most animal blood samples up to the concentration reported in the table above. Positive results were obtained with blood from primates, and of Mustelidae (weasel family). Badger shows a weak positive signal with concentrations of 200 µg/ml. Horse blood is negative at least up to 100 µg/ml. Concentrations of 500 µg/ml show borderline positive results.
Additional animal blood samples will be tested as they become available.

3.2 Interferences

A number of potentially interfering substances have been added to sample transport medium with and without human haemoglobin present.

Substance	Concentration	Test result haemoglobin concentration	
		0 ng/ml	50 ng/ml
Ascorbic acid	40 mg/dl	-	+
Ampicillin	40 mg/dl	-	+
Acetylsalicylic acid	20 mg/dl	-	+
Atropine	40 mg/dl	-	+
Caffeine	40 mg/dl	-	+
Glucose	2.000 mg/dl	-	+
Human albumin	2 mg/dl	-	+
Gentisic Acid	40 mg/dl	-	+
Acetaminophen	20 mg/dl	-	+
Urea	4.000 mg/dl	-	+
Uric acid	10 mg/dl	-	+

- = negative; + = positive

No interferences/cross-reactions could be observed with the tested substances.

4 Accuracy

To evaluate the diagnostic sensitivity and specificity 105 predetermined clinical specimens were tested with HEXAGON OBTI (lot. 14090103).

		HEXAGON OBTI		Total results
		Positive	Negative	
Clinical samples	Positive	36	0	36
	Negative	0	69	69
Total results		63	151	105

Conclusion: HEXAGON OBTI showed in comparison to a commercially available rapid test a diagnostic sensitivity of 100% (36/36) and a diagnostic specificity of 100% (69/69). The accuracy is 100% (105/105).

5. Reproducibility

Specimens
Control specimens containing 50 ng/ml, 100 ng/ml and 200 ng/ml human haemoglobin and confirmed negative clinical specimens were tested.

Between-run, Lot-to-lot
The above mentioned specimens were tested with three different lots.

Results and conclusion: The agreement of all readings was 100%. There were no deviations between the runs and the lots.

6. Stability

6.1 Real time stability results

The stability of HEXAGON OBTI has been demonstrated by real-time stability studies. Three different lots: Lot1 (14090101), Lot 2 (14090102) and Lot 3 (14090103), manufactured in 09/2014 were tested. Function tests of the completed kits have been performed using positive controls with haemoglobin concentrations of 50 ng/ml, 100 ng/ml and 200 ng/ml and negative specimens. Testing at each time point was done in two replicates.

For real-time stability tests, two sets of three different lots of HEXAGON OBTI were stored refrigerated (2-8°C) and at room temperature (15-30°C) for 24 months and tested at different time points (1, 2, 4, 6, 8, 10, 12 14 16, 18, 20, 22 and 24 months) with the above mentioned specimens.

Months	Lot	Test 1				Test 2			
		200 ng/ml	100 ng/ml	50 ng/ml	Negative control	200 ng/ml	100 ng/ml	50 ng/ml	Negative control
1	Lot 1	7	5	4	0	7	5	4	0
	Lot 2	7	5	4	0	7	5	4	0
	Lot 3	7	5	4	0	7	5	4	0
2	Lot 1	7	5	4	0	7	5	4	0
	Lot 2	7	5	4	0	7	5	4	0
	Lot 3	7	5	4	0	7	5	4	0
4	Lot 1	7	5	4	0	7	5	4	0
	Lot 2	7	5	4	0	7	5	4	0
	Lot 3	7	5	4	0	7	5	4	0
6	Lot 1	7	5	4	0	7	5	4	0
	Lot 2	7	5	4	0	7	5	4	0
	Lot 3	7	5	4	0	7	5	4	0
8	Lot 1	7	5	4	0	7	5	4	0
	Lot 2	7	5	4	0	7	5	4	0

	Lot 3	7	5	4	0	7	5	4	0
10	Lot 1	7	5	4	0	7	5	4	0
	Lot 2	7	5	4	0	7	5	4	0
	Lot 3	7	5	4	0	7	5	4	0
12	Lot 1	7	5	4	0	7	5	4	0
	Lot 2	7	5	4	0	7	5	4	0
	Lot 3	7	5	4	0	7	5	4	0
14	Lot 1	7	5	4	0	7	5	4	0
	Lot 2	7	5	4	0	7	5	4	0
	Lot 3	7	5	4	0	7	5	4	0
16	Lot 1	7	5	4	0	7	5	4	0
	Lot 2	7	5	4	0	7	5	4	0
	Lot 3	7	5	4	0	7	5	4	0
18	Lot 1	7	5	4	0	7	5	4	0
	Lot 2	7	5	4	0	7	5	4	0
	Lot 3	7	5	4	0	7	5	4	0
20	Lot 1	7	5	4	0	7	5	4	0
	Lot 2	7	5	4	0	7	5	4	0
	Lot 3	7	5	4	0	7	5	4	0
22	Lot 1	7	5	4	0	7	5	4	0
	Lot 2	7	5	4	0	7	5	4	0
	Lot 3	7	5	4	0	7	5	4	0
24	Lot 1	7	5	4	0	7	5	4	0
	Lot 2	7	5	4	0	7	5	4	0
	Lot 3	7	5	4	0	7	5	4	0

Note: scale 0-10, 0-1: Negative, 2: borderline positive, 3 or above: Positive.

Conclusion: The obtained results of the real-time stability study support the claimed shelf life of 24 months from the date of manufacturing when stored in the recommended temperature range between 2 and 30°C.