

Hemoglobin (HGB) is the main protein in mature red blood cells. It is tetramer and one molecule of hemoglobin in adult is usually composed of four polypeptide chains, 2 alpha and 2 beta. Each of polypeptide chain is linked to a heme molecule. The main function of hemoglobin is to transport oxygen from the lungs to the body's tissues and then transport carbon dioxide out of the tissue back to the lungs.

Colorectal cancer (CRC) is one of the most common causes of death from cancer. However, the success rate of treatment is over 90% when detected at early stages and properly treated. Early detection of CRC is the key of survival. Fecal occult blood (FOB) test have been proven to be the effective tools for CRC screening. Feces with a small amount of blood is the only indications of early CRC, so it has great significance for CRC to detect hemoglobin in human stool samples.

Anti-HGB monoclonal antibodies

Two latest anti-HGB monoclonal antibodies (Catalog Number : CSB-DA438HmN①, CSB-DA438HmN②) have been developed by CUSAg. The sensitivity and specificity of anti-HGB monoclonal antibodies have been repeatedly tested by gold immunochromatography assay (GICA). This product may be sold for in vitro diagnosis.

PROPERTIES	SPECIFICATION
Target species	Human
Host animal	Mice Balb/c
Cell line used for fusion	Sp2/0
Immunogen	Human hemoglobin
Purification method, purity	Protein G affinity chromatography, >90%(SDS-PAGE)
Presentation	MAB solution in PBS with 15 mM NaN ₃ (pH 6.0)
Application	gold immunochromatography assay (GICA)
Catalog Number	CSB-DA438HmN① CSB-DA438HmN②

HGB
HGB

HGB

1 Linearity

The calibrator were spiked with human HGB at 0, 200,500,750 and 1000 ng/mL in saline buffer. The FOB test requirements consists of a pad containing monoclonal anti-HGB (CSB-DA438HmN①) antibodies conjugated to colloidal gold, a nitrocellulose strip containing a test line which contains monoclonal anti-HGB antibodies (CSB-DA438HmN②), and a control line which contains polyclonal anti-mouse IgG antibodies. As same as FOB high-quality comparison kit ,the cut-off was also determined to be 200 ng/mL and no prozone hook effect was observed up to 1000 ng/mL.

2 Clinical analysis

10 Stool extract samples (H1-H10) from donors with negative diagnoses were tested with the predicate and the test device. There was 100% agreement between the high-quality comparison kit (A) and immunoassay using CUSAg anti-HGB antibodies (B) for the negative samples. Each of stool extract samples were spiked with human HGB at 500 ng/mL for positive samples (P1-P10) .Two test device results were positive. The positive percent agreement was 100%.

Table1. Clinical comparison of diagnostic kit (A) and immunoassay using CUSAg anti-HGB antibodies (B).

samples methods	H1	P1	H2	P2	H3	P3	H4	P4	H5	P5	H6	P6	H7	P7	H8	P8	H9	P9	H10	P10
A	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
B	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+

"+" : positive, "-" :negative. "A" :high-quality kit, "B" :immunoassay using CUSAg anti-HGB antibodies (B).

3 Specificity

The immunoassay using CUSAg anti-HGB antibodies is specific to human HGB. When the positive and negative samples concentration is below the level of the material shown in the following list, the following substances does not affect the test results.

Table2. Specificity of CUSAg anti-HGB antibodies

Substance	Concentration(µg/ml)
Beef Hemoglobin	2000
Fish Hemoglobin	100
Chicken Hemoglobin	500
Pig Hemoglobin	500
Rabbit Hemoglobin	500
Red radish	Aqueous extract
Vegetables	Aqueous extract
Iron	Dietary supplement

4 Thermal Stability

CUSAg anti-HGB antibodies were respectively stored at -20°C, 2-8°C and 37°C for 14 days. negative samples(A1-E1), positive samples (A2-E2) and calibrator were prepared. After then, these samples were detected, respectively (Table 3). The results show that the stability of CUSAg anti-HGB antibodies is perfect.

Table3. Thermal stability of CUSAg anti-HGB antibodies

temperature samples	-20°C	2-8°C(14 days)	37°C(14 days)
0 ng/mL	-	-	-
200 ng/mL	+	+	+
500 ng/mL	+	+	+
750 ng/mL	++	++	++
1000 ng/mL	+++	+++	+++
A1	-	-	-
B1	-	-	-
C1	-	-	-
D1	-	-	-
E1	-	-	-
A2(500 ng/mL)	+	+	+
B2(500 ng/mL)	+	+	+
C2(500 ng/mL)	+	+	+
D2(500 ng/mL)	+	+	+
E2(500 ng/mL)	+	+	+

"+" : general positive, "++" : positive, "+++ " : intense positive. "-" : negative.

References

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- 4.Christopher S. Thom, Claire F. Dickson,David A. Gell.Hemoglobin Variants: Biochemical Properties and Clinical Correlates. CSH. 2013 Mar; 3(3): a011858.

WUHAN HUAMEI BIOTECH CO.,LTD © CUSAg IVD RAW MATERIALS DIVISION

ADDR: No.818 Gaoxin Avenue, Wuhan Hi-tech Medical Devices Park, Donghu High-tech Development Zone 430206, Wuhan City, Hubei Province, P.R. China.

TEL: +86-27-87196282 Ext.837/853 FAX: +86-27-87196150

EMAIL: cusag@cusag.cn WEB: www.cusag.org

