

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 | 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 | -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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