

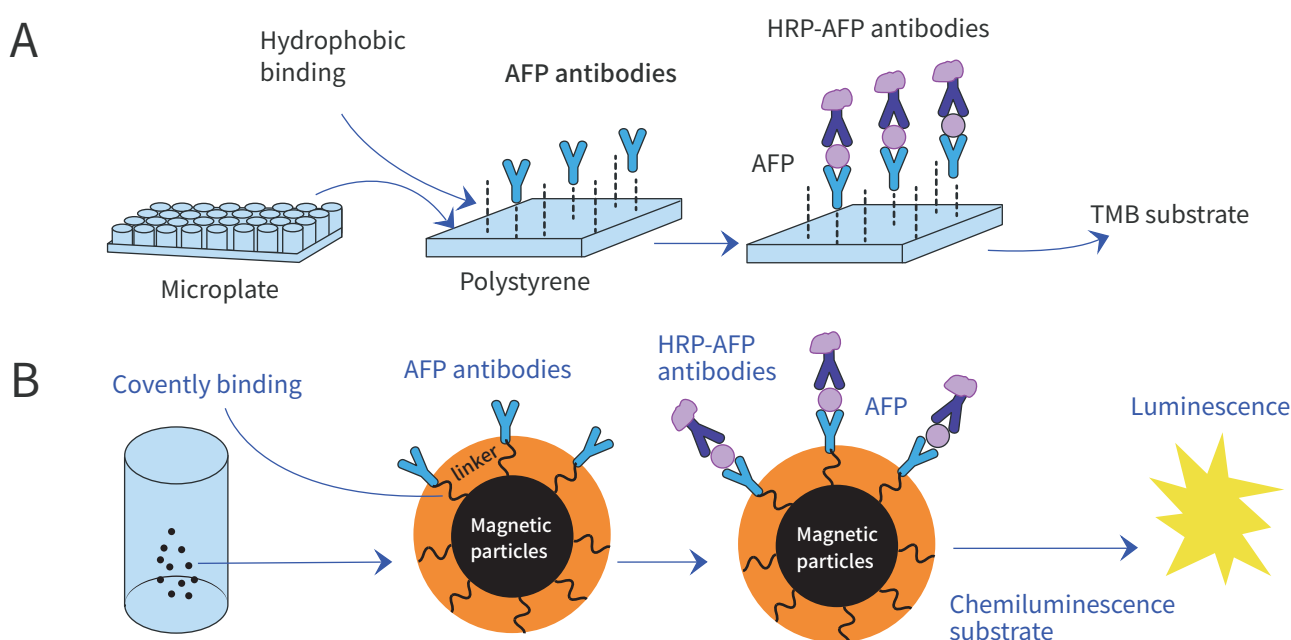
Application of magnetic microspheres in the chemiluminescence platform of alpha-fetoprotein (AFP) detection

Background:

Hepatocellular carcinoma (HCC) is the main histological subtype of liver cancer, accounting for 90% of primary liver cancer, and is the third most common cause of cancer-related mortality worldwide. The main risk factors for HCC are chronic infection with hepatitis B and C viruses (HBV and HCV), long-term alcohol consumption, and the metabolic changes leading to steatosis and non-alcoholic steatohepatitis (NASH) discovered in recent years. In practice, HCC screening is usually required for patients exposed to the various risk factors for HCC, especially when cirrhosis is present. Screening mainly relies on liver ultrasound and imaging. However, tumor marker analyses are less subjective, and potentially more sensitive than imaging diagnosis, and receive more extensive research and attention. AFP is the main serological marker used in the diagnosis of HCC, since it is secreted by about half of HCC tumors. AFP plasma concentration of 20ng/mL is usually used as the pathological threshold for humans, and the concentration higher than 400 ng/mL is usually considered as a reliable indicator for the diagnosis of HCC. Therefore, in the clinical diagnosis and prognosis of liver cancer, sensitive and time-saving AFP detection is very necessary.

Advantages of Magnetic Particles Based Chemiluminescence Detection of AFP

Use magnetic particles-based CLEIA (MPs-CLEIA) to detect AFP in human serum in parallel with ELISA. The experimental results show that MPs-CLEIA has significantly advantages over ELISA in terms of low dosage of immune reagents, high dose hook effect and high biological activity of immune reagents, short detection time, and wide linear range. MPs-CLEIA detection of AFP can be considered as a convenient, economical, and time-saving HCC screening and prognostic detection method.



The principle of detecting AFP in human serum based on HRP a: ELISA b: magnetic particles-based chemiluminescence

1. Experimental method:

The alpha-fetoprotein quantitative detection kit was used to compare the stability, precision and reactivity of VDO Biotech's magnetic microspheres and Company J's magnetic microspheres at 37°C.

2. Material and reaction system:

(1) Standard: AFP

(2) Reaction mode: one-step reaction

(3) Magnetic microspheres: CMP1003CA (VDO Biotech) compared to Company J's magnetic microspheres

(4) Coated antibody: 20µg antibody/mg-beads

Labeled antibody: the molar ratio of antibody/acridinium ester is 1/20

(5) Process: add 25µL sample, 100µL acridinium ester, and 40µL streptavidin magnetic microspheres into a tube, incubate at 37°C for 20 minutes, and then wash 5 times.

3. Result:

(1) Stability test at 37°C

The magnetic microspheres CMP1003CA (VDO Biotech) and Company J magnetic microspheres were coated with antibodies and divided into 2 parts separately. One part was placed at 2-8°C, and the other part was placed at 37°C. The signal intensity was tested on the 8th day.

According to the following results, under the same experimental conditions, the signal intensity of VDO magnetic microspheres is basically the same as that of Company J magnetic microspheres, and the stability of VDO magnetic microspheres is equivalent to that of Company J magnetic microspheres.

	Company J's Magnetic Microspheres			VDO Magnetic Microspheres		
Antigen concentration (ng/mL)	2-8°C	37°C	Deviation	2-8°C	37°C	Deviation
0	8787	8621	-1.9%	8061	7972	-1.10%
5	99936	97532	-2.4%	95505	93117	-2.50%
25	406235	389985	-4.0%	380296	365844	-3.80%
50	80904	779112	-3.7%	760986	730546	-4.00%
200	2853867	2786632	-2.4%	2601184	2520547	-3.10%
600	6519703	6193717	-5.0%	6430896	6147936	-4.40%

Stability test data at 37°C

(2) Precision test of magnetic microspheres

Select antigen concentration of 0, 5, 200ng/mL, and test 6 times for each concentration. The results are as follows, which proves that the VDO magnetic microspheres are comparable in precision and repeatable compared with the Company J magnetic microspheres.

	Company J's Magnetic Microspheres			VDO Magnetic Microspheres		
	0	5	200	0	5	200
Signal strength	8827	99589	2963576	8034	93379	2613098
	8828	106897	2959311	8031	93718	2625467
	8985	108729	2830556	8154	93889	2519362
	8613	109310	2958690	8274	93771	2570575
	8795	101607	2846978	8040	93287	2678410
	8890	100544	2730907	8188	93659	2687760
AVERAGE	8823	104446	2881669.667	8120.166667	93617.16667	2615778.667
STDEV	123.0105687	4356.281901	95094.11774	101.2055664	234.6098179	64100.84419
CV	1.39%	4.17%	3.30%	1.25%	0.25%	2.455%

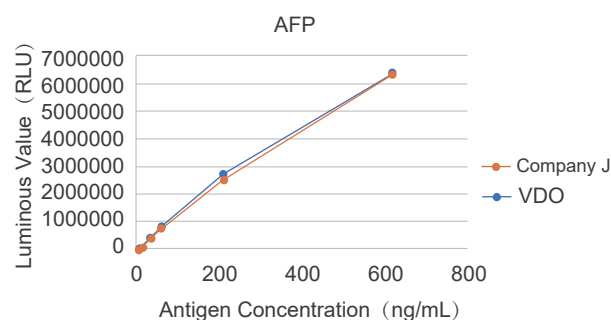
Precision test of magnetic microspheres

(3) Reactivity test of magnetic microspheres

Test the reactivity of each antigen concentration of 0, 5, 25, 50, 200, 600 ng/mL. Under the same conditions, when the antigen concentration is 0ng/mL, VDO's magnetic microspheres shows less interference; with other antigen concentrations, the signal strength of VDO's and Company J's magnetic microspheres are comparable.

Detection of alpha-fetoprotein (AFP) by magnetic microsphere chemiluminescence method

Antigen Concentration	Company J's Magnetic Microspheres	VDO's Magnetic Microspheres
0ng/mL	8787	8061
5ng/mL	99936	95505
25ng/mL	406235	380296
50ng/mL	809104	760986
200ng/mL	2853867	2601184
600ng/mL	6519703	6430896



Reactivity test of magnetic microspheres

4. Conclusion:

In the AFP test, VDO Biotech's streptavidin magnetic microspheres and Company J magnetic microspheres have comparable test results on key indicators such as stability, precision, and signal intensity, and are suitable for this test.

Reference:

1. Sauzay C, Petit A, Bourgeois A M, et al. Alpha-foetoprotein (AFP): A multi-purpose marker in hepatocellular carcinoma[J]. Clinica chimica acta, 2016, 463: 39-44.
2. Zhang Q Y, Chen H, Lin Z, et al. Comparison of chemiluminescence enzyme immunoassay based on magnetic micro-particles with traditional colorimetric ELISA for the detection of serum α -fetoprotein[J]. Journal of pharmaceutical analysis, 2012, 2(2): 130-135.

VDO Biotech Magnetic Microspheres for Chemiluminescent

VDO Biotech's magnetic microspheres for chemiluminescent have superparamagnetism and moderate magnetic content, excellent resuspendability and fast magnetic response. With our advanced microsphere synthesis technology, proprietary surface coating process, and variety selections of functional groups, our magnetic microspheres provide comprehensive solutions to meet customers' specific needs of different technology route development. The high-load functional group guarantees the binding capacity, and this series of products show outstanding performance in the field of immunoassay.



Cat. No.	Particle Size	Color	Surface Groups	Solids	Size
CMP0600CA	0.6 μ m	Brownish yellow	COOH	2.5%	10ml, 100ml, 1L
CMP1001CA	1 μ m	Brownish yellow	COOH	2.5%	10ml, 100ml, 1L
CMP1003CA	3 μ m	Brownish yellow	COOH	2.5%	10ml, 100ml, 1L
CMP0600SA	0.6 μ m	Brownish yellow	SA	1.0%	10ml, 100ml, 1L
CMP1001SA	1 μ m	Brownish yellow	SA	1.0%	10ml, 100ml, 1L
CMP1003SA	3 μ m	Brownish yellow	SA	1.0%	10ml, 100ml, 1L



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