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Rapid Detection of HIV-1 p24 antigen by magnetic immuno-chromatographic testing (MICT)

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Abstract

Objective: To develop a sensitive and rapid HIV assay for the detection of HIV-1 p24 antigen in serum/plasma. This assay could be used in low volume venues to improve early detection of HIV infection in adults and newborns.

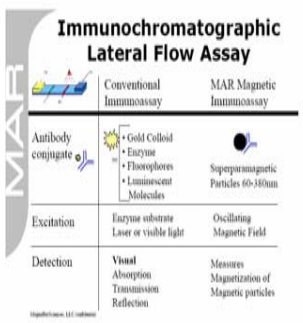
Methods: MICT uses standard lateral flow assay technology assay with super-paramagnetic particles as the detection reagent. Monoclonal (mouse) and polyclonal (rabbit) antibodies to HIV-1 p24 protein were produced, purified and characterized. Polyclonal capture of p24 was detected using a mouse monoclonal (1E5 directed to the C terminus of p24) covalently conjugated to 300 nm super paramagnetic particles. The formation of the immune complex at the test line is measured by the Magnetic Assay Reader (MAR) from MagnaBiosciences. The reader uses magnetic sensors to create a reaction curve, which is mathematically validated and integrated, versus an ideal magnetic spectrum. The amount of p24 detected is directly proportional to the area under the curve. Assay performance was determined using p24 and viral-spiked buffers and plasma, and p24 reactive specimens from HIV seroconversion panels.

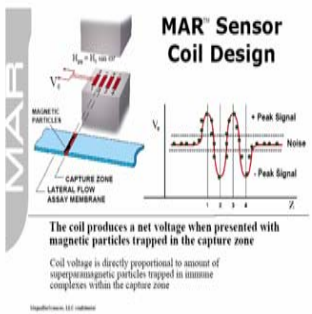
Results: MICT assay showed reproducible detection of ~25pg/mL of HIV p24 antigen in running buffer. Detection of p24 spiked plasma was initially reduced by 75% due to matrix effects. Buffer modifications and a reduction of plasma to 50% significantly improved detection (assay sensitivity of 62 pg/ml). P24 was detectable in virus-spiked buffers and in seroconversion panels at high viral concentrations.

Conclusions: MICT detects HIV p24 antigen at concentrations slightly higher than conventional EIAs but in much less time (30 min vs. 2.5 hr). Further optimization of the assay is underway to reduce matrix effects and to improve the lower limit of detection

Purpose: To develop a simple, rapid lateral flow assay for the quantitative detection of HIV-1 p24 antigen in serum and plasma specimens using MICT.

Background

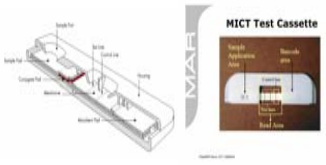




Materials and procedures

Materials

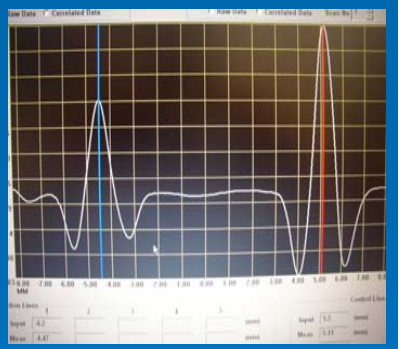
- Rabbit polyclonal antibodies to HIV-1 p24 protein
- Mouse monoclonal antibodies to HIV-1 p24 protein
- Lateral flow cassette format



- Running buffer
- Monoclonal antibody conjugated to super paramagnetic particles in solution
- Magnetic Assay Reader (MAR) from MagnaBiosciences

Procedures

- Specimen is mixed with paramagnetic particle solution
- incubate for 2 minutes at room temperature
- Transfer 100 uL of the mixture solution into the sample port on the test cassette
- Read test cassette for MAR value at 20, 30 and 40 minutes.



Graphical depiction of paramagnetic particle detection in oscillating magnetic field

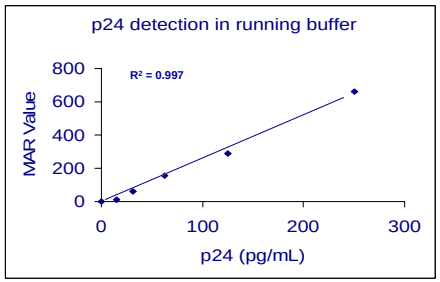
Quantitative Measurement (MAR value)

- Three magnetic field detections
- Super paramagnetic particles are magnetic only when in a magnetic field
- This allows them to be freely manipulated in solution without aggregating
- Field distortion is directly proportional to the mass of magnetic particles present
- Measures total particles trapped not just ones on the top of the membrane
- The amount of p24 detected is directly proportional to the area under the curve
- Curve fit values > 0.90 are considered significant

Results

HIV-1 p24 detection of p24 antigen spiked into running buffer

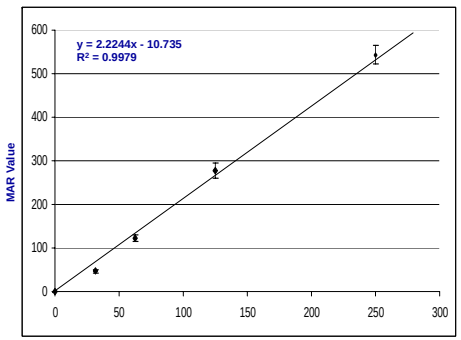
	20 minutes				30 minutes			
	Control		Test		Control		Test	
P24 (pg/mL)	MAR	Fit	MAR	Fit	MAR	Fit	MAR	Fit
250	340.2	**0.9655**	390.1	**0.9888**	520.5	**0.9653**	663.1	**0.9885**
125	241.5	**0.9621**	159.4	**0.9880**	365.8	**0.9646**	288.5	**0.9898**
62.5	342.4	**0.9667**	76.0	**0.9869**	507.9	**0.9650**	153.9	**0.9851**
31	309.9	**0.9666**	**23.7**	**0.9590**	464.2	**0.9643**	**58.7**	**0.9662**
15.6	330.3	**0.9641**	0	0.2738	504.6	**0.9647**	11.3	0.7589
0	329.3	**0.9634**	0	0.1231	474.6	**0.9660**	0	0.0913



Detected 31 pg/mL of p24 as early as 20 minutes

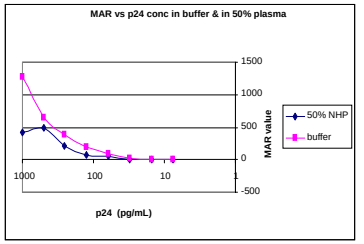
Reproducible detection of p24 antigen spiked into running buffer

No.	250 pg	125 pg	62.5 pg	31.25 pg	0
1	506.0	245.3	118.1	**37.6**	0
2	645.0	361.5	148.4	68.8	0
3	551.5	271.7	126.7	**53.1**	0
4	466.8	234.2	119.3	**56.8**	0
5	453.0	215.3	97.3	**34.5**	0
6	499.8	231.5	110.9	**42.5**	0
7	490.9	227.5	90.1	**27.2**	0
8	601.1	367.4	120.4	**54.4**	0
9	520.1	250.9	89.3	**24.8**	0
10	663.1	288.5	153.9	**58.7**	0
11	582.8	353.3	167.7	**51.5**	0
ST DEV	70.9	57.6	25.8	14.0	0
AV	543.6	277.0	122.0	46.4	0
CV	13.0	20.8	21.2	30.2	0



Detection of p24 antigen spiked into running buffer and into 50% plasma

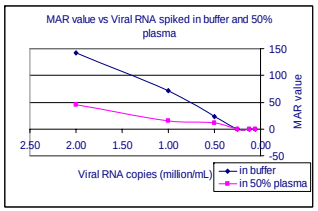
pg/mL p24	buffer	50% plasma
1000	1280	424.9
500	650.8	488.9
259	380.6	213.3
125	199.1	78.7
62	86.2	**50.1**
31	**27.9**	**11.6**
15	3.7	0
7.5	8.5	0
0	0	0



Detected 31 pg/mL p24 spiked in buffer
Detected 62 pg/mL p24 spiked in 50% plasma

p24 detection of HIV-1 virions spiked into running buffer and into 50% plasma

Viral RNA copies x 10^6	p24 (pg/mL)	running buffer	50% plasma
2.00	80	141.9	45.1
1.00	40	71.2	15.1
0.50	20	23.5	12
0.25	10	0	0
0.13	5	0	0
0.06	2.5	0	0



Detected 0.5 x 10^6 viral RNA copies/mL (~20 pg/mL) spiked in buffer
Detected 1.0 x 10^6 viral RNA copies/mL (~40 pg/mL) in 50% plasma

HIV-1 seroconversion panel specimen diluted 1:2 in running buffer

Specimen member	Viral RNA copies/mL	p24 (pg/mL)	MAR value
6248-05	2.3 x 10^3	0.08	0
6248-06	2.0 x 10^5	8.00	12.4
6248-07	8.7 x 10^7	3480.00	618.4

Relationship of p24 mass to viral RNA copies

- 100 pg = 2.5 X 10^6 RNA copies
- 10 pg = 2.5 X 10^5
- 1 pg = 2.5 X 10^4

- 1 pg = 2.5 X 10^7 molecules p24

Conclusions

Benefits of using Rapid Detection of HIV-1 p24 antigen by MICT

- Early detection of HIV infection in adults and newborns
- Low sample volume required
- Detection of HIV-1 p24 antigen can be measured as early as 20 minutes
- Measurement of each analytical region performed in ~30 seconds
- Quantitative results provided
- Less sensitive than conventional EIAs but much lower cost
- Further MICT assay optimization needed for patient samples

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