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Detection of antibodies to HIV-1 and HIV-2 by rapid magnetic immuno-chromatography testing (MCIT): Effectiveness of multi-subtype (HIV-1) and branched peptide (HIV-2) antigens to differentiate HIV infections.

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ABSTRACT

Detection of antibodies to HIV-1 and HIV-2 by rapid magnetic immuno-chromatography testing (MICT): Effectiveness of multi-subtype (HIV-1) and branched peptide (HIV-2) antigens to differentiate HIV infections. S K Wells, S Workman, C-P Pau, and T C Granade. Centers for Disease Control and Prevention, Atlanta, GA.

Objectives: The purpose of this study was to evaluate rapid magnetic immuno-chromatography technology for use in detecting antibodies to HIV-1 and HIV-2 and to determine the effectiveness of recombinant multi-subtype (HIV-1) and branched peptide (HIV-2) antigens to detect and to differentiate HIV specific antibodies.

Methods: Magnetic immuno-chromatography testing (MICT) may be formatted as a traditional lateral flow assay using magnetic particles as the detector in place of colloidal gold or other colored markers. Analytes of interest are detected using a small instrument capable of quantitatively measuring distortions in the magnetic field associated with the specific capture of conjugated magnetic particles. We developed an MICT for the detection of antibodies to HIV-1 and HIV-2 and evaluated the MICT performance with a set of 370 serum/plasma specimens from the US, Cameroon, and the Ivory Coast (200 non-reactive, 90 HIV-1, 28 HIV-2, and 7 HIV-1 seroconversion panels [n=52]). Panel members were also tested by enzyme immunoassay (EIA)/ Western blot (WB) [reference standard], a commercial rapid test (OraQuick), and an in-house rapid lateral flow colloidal gold assay comparable to the MICT.

Results: MICT detection is based on relative magnetic units (RMU). Non-reactive specimens averaged 17.5 and 2.6 RMU for the HIV-1 and HIV-2 antigens, respectively. A cutoff value for each antigen was established by adding 4 standard deviations resulting in 55 RMU for HIV-1, 30 RMU for HIV-2. No false positive reactions were noted (specificity = 100%). The results of the HIV-1 specimens and HIV-1 seroconversion panel members were congruent with EIA/WB, OraQuick, and the in-house rapid test results (sensitivity = 100%). Average RMU for strongly reactive specimens was 1660. All 28 HIV-2 specimens were detected (average RMU= 550), however, two specimens were classified as HIV-1 by MICT due to cross-reactivity.

Conclusions: MICT is effective in identifying HIV antibodies in serum and plasma. The HIV-1 and HIV-2 antigens effectively captured HIV specific antibodies; however, additional optimization of the MICT assay and antigen deposition could improve the quantitative nature of the test. MICT could be effective at identifying early infection especially if configured as a third generation format.

Objectives

- To evaluate rapid magnetic immuno-chromatography technology for use in detecting antibodies to HIV-1 and HIV-2.
- To determine the effectiveness of recombinant multi-subtype (HIV-1) and branched peptide (HIV-2) antigens to detect and to differentiate HIV specific antibodies.

Methods

- Selected buffers, membranes and pads to be used for the assay.
- Preparation of HIV-1 recombinant multi-subtype peptide and HIV-2 branched peptide
- Prepared colloidal gold conjugate and protein A magnetic beads.
- Striped peptides and protein A as a control line onto membranes for both the gold lateral flow assay and the MCIT assay.
- Ran the gold assay first to make sure the peptides would work in lateral flow format.
- The lateral flow assay worked so next the MCIT assay was run.

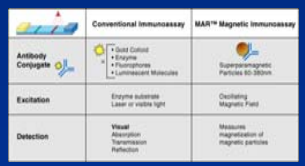
Ideal Characteristics for Rapid Diagnostic Assays

- Reading frame of 15-20 minutes
- Simple to perform and easy to interpret
- Few reagents to reconstitute or manipulate
- Sample preparation is simple and quick
- No expensive equipment required

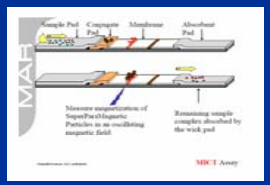
MCIT BACKGROUND

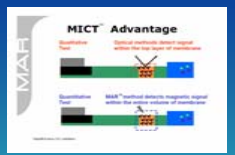
- Magnetic Nanoparticles have been used in the medical field for several years for nucleic acid separation, detection, as MRI contrast agents and other applications
- Colloidal paramagnetic particle labels utilize the the ability of antibodies to link the analyte of interest to the magnetic nanoparticle.
- The analyte is then labeled with the particles.
- With the application of a magnetic field, the isolation or separation is performed.
- Magnetic particles can be substituted for colloidal gold in lateral flow assays.
- A magnetic assay reader (MAR) is used to obtain the results. The signal is amplified to give a value that that indicates the quantity of magnetic particles detected.

Comparison of Lateral Flow and MT



MICT Cassette Design





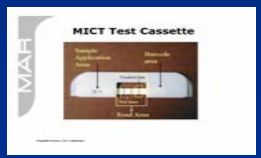
Gold Assay Format

Control line (protein A), HIV-1 peptide, HIV-2 Peptide
Colloidal Gold Conjugate pad
1ul of specimen added to 200ul buffer
Add strip to tube
Read at 20 minutes and 40 minutes

Colloidal Gold Assay Strips

MICT Assay Format

Control line (protein A), HIV-1 peptide, HIV-2 peptide
Protein A conjugated to 300 nm beads
1 ul of specimen added to 200 ul buffer
Add 2 ul of Protein A-MB
Add 100 ul to cassette
Read at 20 minutes



MAR READERS

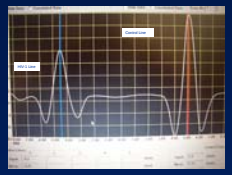
Three sensor magnetic field detection
Field distortion is directly proportional to the mass of magnetic particles present.
Measures total particles trapped not just ones on the top of the membrane.
Improved sensitivity
B Lateral flow design
Hand-held, battery operated instrument
Units are expressed as relative magnetic units (RMU)
Units may be referenced against control line

For Research

Bench Top Diagnostic

Future Handheld

Data from MAR Reader



MAR Sample Readings

Cut/Off
We selected the average negative value + 4 standard deviations which equaled to 55 Mar.

SAMPLE	CONTROL MAR	MAR-1	MAR-2
Negative	1504.1	0	0
Negative	1814.9	9.7	0.6
Negative	1601.3	10.3	0
HIV-1	1377.3	552.3	0
HIV-1	1056.7	1222.5	0
HIV-1	1004.8	466.2	2.6
HIV-2	1188.8	30.1	209.1
HIV-2	2205	24.6	376.1
HIV-2	1123.7	11.5	532.5

Colloidal Gold and MICT Results For Positives and Negatives

HIV Positives and Negatives		OraQuick		Gold		MICT	
		P	N	P	N	P	N
EIA/WB	P 84	83	1	84	0	84	0
	N 182	3	179	2	180	0	182

OraQuick
Sensitivity = 98.8%
Specificity = 98.3%

Gold
Sensitivity = 100%
Specificity = 98.9%

MICT
Sensitivity = 100%
Specificity = 100%

HIV-2 Data

EIA/WB P- 28	EIA/WB N- 0	OraQuick P	OraQuick N	Gold P	Gold N	MICT P	MICT N
28	0	28	0	28	0	28	0

BBI Seroconversion Panel Results

BBI Panel	BBI P	BBI N	WB P	WB N	OraQuick P	OraQuick N	Gold P	Gold N	MCIT P	MCIT N
1	1	5	1	5	1	5	1	5	1	5
2	2	2	1	3	2	2	2	2	2	2
3	4	2	4	2	4	2	4	2	4	2

Conclusions

- MICT is effective in identifying HIV antibodies in serum and plasma.
- Additional optimization of the MICT assay and antigen deposition could improve the quantitative nature of the assay.
- MICT could be effective at identifying early infection especially if configured as a third generation format.

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