Simultaneous Detection of Hepatitis B, C, and HIV Using Multiplo Rapid HBc/HIV/HCV Antibody Test

Neeraj Vats, Ph.D., Karen Black, Ph.D., Pamela Beattie, B.Sc., Jeanna MacLeod, B.Sc., and Carlina Hui, B.Sc., MedMira Laboratories Inc. Halifax, Nova Scotia, Canada

Background

A multiplex test for HBV, HIV, and HCV would be an advantageous tool to maximize resources in providing diagnostic test results to individuals at risk for infection with HBV, HIV, and HCV. HBV, HIV, and HCV share common risk factors and modes of transmission, but are currently diagnosed through distinct programs during unique patient visits. This study assessed a multiplex point-of-care (POC) test, Multiplo Rapid HBc/HIV/HCV Antibody Test (Multiplo HBc/HIV/HCV), in simultaneously detecting early infection. Multiplo HBc/HIV/HCV is a manually performed, visually interpreted immunoassay which detects antibodies (IgG and IgM) to HIV, HBc, and HCV in serum, plasma, and whole blood (venipuncture and fingerstick). Multiplo HBc/HIV/HCV was developed and is manufactured by MedMira Laboratories.

Methods

Panel formation and blinding: Panel members from sixty-nine seroconversion panels obtained from U.S.-based commercial vendors were separated into 27 blinded Panels for testing with Multiplo HBc/HIV/HCV. The identity, and the reactivity, of the samples was blinded from the operators. The blinding was completed by a study coordinator who did not complete functional testing using the Panels. The blinded Panels were created for testing such that each panel member was independently tested by three different operators.

Panel Testing: Testing of the Panels was completed by three operators using one lot of Multiplo HBc/HIV/HCV. Each operator tested Panels provided to them according to the procedure in the Multiplo HBc/HIV/HCV package insert (Fig. 1 and Fig. 2). A set of test controls (one negative and one positive for anti-HBc, anti-HIV and anti-HCV) was tested by each operator on each testing day prior to Panel testing. Test controls were utilized as per the package insert.

Fig. 1 Whole Blood Test Procedure

Pour the entire contents of the sample tube (five (5) drops of Universal Buffer and one (1) drop of whole blood) into the center of the test cartridge. Allow the specimen to absorb completely.

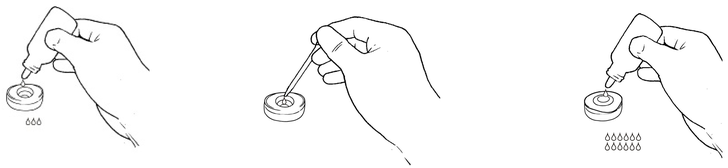
Place the InstantGold cap on the test cartridge.

Dispense twelve (12) drops of Universal Buffer onto the InstantGold cap and allow the solution to absorb completely.

Remove the InstantGold cap, wait for the solution to absorb completely. Add three (3) drops of Universal Buffer to clarify results.

Read test results immediately.

Fig. 2 Serum/Plasma Test Procedure



Apply three (3) drops of Universal Buffer to the center of the test cartridge. Allow the buffer to absorb completely.

Apply one (1) drop of serum or plasma specimen to the center of the test cartridge. Allow the specimen to absorb completely.

Place the InstantGold cap on the test cartridge.

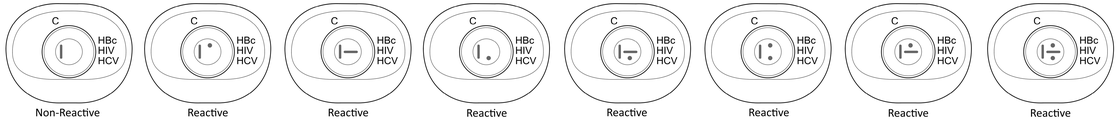
Dispense twelve (12) drops of Universal Buffer onto the InstantGold cap and allow the solution to absorb completely.

Remove the InstantGold cap, wait for the solution to absorb completely. Add three (3) drops of Universal Buffer to clarify results.

Read test results immediately.

Test results for test controls and panel members were interpreted according to the package insert (Fig. 3). Each operator recorded all test control and Panel Member test results on supplied Panel Testing Record forms. Upon completion of testing, the Panel Testing Record forms were forwarded to the study coordinator for decoding and compilation of data. The average days to detect seroconversion was calculated relative to the results of FDA-approved laboratory-based immunoassays reported by supplier of the seroconversion panels.

Fig. 3 Test Results Interpretation



Results

Results were recorded for each seroconversion panel member, one result for each operator that independently tested the blinded panels. In most cases, concordant results were obtained (data not shown) across the three operators. The summary analysis of anti-HBc, anti-HCV, or anti-HIV antibodies per seroconversion panel is detailed in Tables 1-3. Multiplo HBc/HIV/HCV performed within expectations; seroconversion detection, as evidenced by detection of anti-HBc, anti-HCV, or anti-HIV antibodies, by Multiplo HBc/HIV/HCV occurred on the same bleed, or within one bleed as the laboratory-based immunoassay consensus for 56 of the 61 (92%) seroconversion panels included in the analysis. Of the remaining five seroconversion panels, Multiplo HBc/HIV/HCV detected seroconversion within four bleeds. Per marker, Multiplo HBc/HIV/HCV detected seroconversion on average 3.7 days later for HBc, 0.4 days later for HIV, and 0.7 days later for HCV. The later HBc detection may be due to; fewer panels being assessed; 9 HBV panels vs. 30 for each of HIV and HCV, and the number of days between bleeds for the panels that were available.

Table 1. Seroconversion sensitivity of Multiplo HBc/HIV/HCV for anti-HBc antibody detection, as compared to a consensus of FDA approved assays

Panel Lot Number	Initial HBc Antibody Detection (Days from first bleed)		Difference in Days to HBc Antibody Detection
	Comparator Assay(s)[1]	Multiplo HBc/HIV/HCV[2]	
PHM933(M)	144	144	0
PHM935B	128[3]	128[3]	N/A[3]
6278	41	41	0
6281	41	43	+2
9072	154	Not detected (160)[4]	>5 (6)[4]
9092	85	92	+7
9093	42	49	+7
10231	N/A[5]	N/A[5]	N/A[5]
10232	N/A[5]	N/A[5]	N/A[5]
Total days	507/6 = 84.5	529/6=88.2	22/6=3.7

[1] Based on the reactivity of FDA approved laboratory-based immunoassays for anti-HBc detection. If only 1 comparator assay was available, it was considered sufficient use as a comparator assay. If two or more comparator assays were available, 2 assays had to be reactive to achieve consensus.
[2] Based on first reactive result as concordant across all three operators.
[3] No negative bleed was available for this panel. Panel began at 128 days from first bleed and therefore does not adequately represent the first detection of anti-HBc by comparator assay or Multiplo HBc/HIV/HCV. Data have not been included in overall calculations. Panel was included in evaluation to illustrate the comparability to anti-HBc laboratory-based immunoassay.
[4] No panel members in seroconversion panel lot 9072 provided reactive results on Multiplo HBc/HIV/HCV. For the purpose of calculations, 160 days was used for the Multiplo HBc/HIV/HCV detection and 6 days was used as the difference in days to anti-HBc antibody detection; this represents one day after the last bleed date of the final panel member.
[5] Anti-HBc was not detected by comparator assay nor by Multiplo HBc/HIV/HCV. Data are not included in overall calculations of seroconversion sensitivity. Panels were included in evaluation to illustrate the comparability to anti-HBc laboratory-based immunoassay.

Table 2. Seroconversion sensitivity of Multiplo HBc/HIV/HCV for anti-HCV antibody detection, as compared to consensus of FDA approved laboratory-based immunoassays

Panel Lot Number	Geno-type, if available	Initial HCV Antibody Detection (Days from first bleed)		Difference in Days to HCV Antibody Detection
		Comparator Assay Consensus[1]	Multiplo HBc/HIV/HCV[2]	
PHV911(M)	1a	14	14	0
PHV912	2b/3	7	7	0
PHV913	2b	7	7	0
PHV914	2b	24	12	-12
PHV915	2b	12	12	0
PHV917(M)	2b	85	85	0
PHV918(M)	1a	24	24	0
PHV919	1a	28	28	0
PHV921	3a	4	4	0
PHV922	3a	3	3	0
PHV923	1a	21	21	0
6215	1a	20	20	0
6223*	N/A	Not applicable[3]	Not detected	N/A
6225	N/A	78	78	0
6226	N/A	37	37	0
6227	N/A	74	74	0
6228	1a	28	36	+8
6229	N/A	17	28	+11
9041	N/A	62	62	0
9042*	N/A	Not applicable[3]	Not detected	N/A
9044	N/A	25	25	0
9045	N/A	37	41	+4
9046	N/A	69	69	0
9047	N/A	28	28	0
9055	3a	Not detected[4]	Not detected	N/A
9058	1a	10	14	+4
10039	N/A	Consensus not reached[5]	Not detected	N/A
10041	N/A	6	10	+4
10057	1a	152	152	0
10062	N/A	41	41	0
Total days		913/26[6] = 35.1 days	932/26[6] = 35.8 days	19/26[6] = 0.7 days

*False positive seroconversion panel.
[1] Based on the consensus reactivity of FDA approved laboratory-based immunoassays for anti-HCV detection. If only 2 comparator assays were available, both needed to be reactive to achieve consensus. If three or more comparator assays were available, 2 assays had to be reactive to achieve consensus.
[2] Based on attainment of concordant results by the three operators upon independent testing of blinded panels.
[3] While comparator assay consensus was achieved for anti-HCV detection, all panel members are non-reactive for HCV nucleic acid and thus will not be included in the seroconversion sensitivity calculations.
[4] FDA approved comparator assays for anti-HCV detection were non-reactive for all panel members.
[5] Reactive result provided by Ortho Anti-HCV 3.0 only. Other Anti-HCV Antibody assays were nonreactive. Consensus was not reached.
[6] Totals calculated do not include panels in which Multiplo HBc/HIV/HCV did not detect HCV antibodies.

Table 3: Seroconversion sensitivity of Multiplo HBc/HIV/HCV for anti-HIV antibody detection, as compared to consensus of FDA approved laboratory- based immunoassays

Panel Lot Number	Days to HIV Antibody Detection (Days from first bleed)		Difference in Days to HIV Antibody Detection
	Comparator Assay Consensus[1]	Multiplo HBc/HIV/HCV[2]	
PRB925	44	44	0
PRB943	19	19	0
PRB945	13	15	+2
PRB951	19	19	0
PRB955	12	12	0
PRB958	15	15	0
PRB965	12	12	0
PRB966	48	48	0
PRB967	17	17	0
PRB968	26	26	0
PRB969	70	70	0
PRB970	10	10	0
PRB971	11	11	0
PRB972	18	18	0
PRB973	11	11	0
PRB974	Not Detected[3]	Not detected[3]	N/A
PRB977	15	13	-2
6247	Consensus not reached (31)[4]	30	-1
9019	38	38	0
9022	32	32	0
9027	17	Not detected (29)[5]	>11 (12)[5]
9030	54	54	0
9031	146	146	0
9075	33	33	0
9076	67	67	0
9079	47	47	0
9081	24	24	0
9089	24	20	-4
9096	18	18	0
12007	119	124	+5
Total days	1010/29 = 34.8 days	1022/29 = 35.2 days	12/29 = 0.4 days

[1] Based on the consensus reactivity of FDA approved laboratory-based immunoassays for anti-HCV detection. If only 2 comparator assays were available, both needed to be reactive to achieve consensus. If three or more comparator assays were available, 2 assays had to be reactive to achieve consensus.
[2] Based on first reactive result as agreed by all three operators.
[3] Reactive result provided only by Siemens Advia Centaur, therefore reactive consensus not achieved. Entire panel was non-reactive by other HIV antibody tests and Western Blot and therefore the consensus is negative. Panel was non-reactive by Multiplo HBc/HIV/HCV. Results will not be included in the seroconversion sensitivity calculations.
[4] Reactive result provided only by Abbott Anti-HIV rDNA, therefore reactive consensus not achieved. Entire panel was non-reactive by other FDA-approved HIV antibody tests and Western Blot and therefore consensus is negative. For the purpose of calculations, 31 days was used for the comparator assay detection and -1 days was used as the difference in days to HIV antibody detection, this represents one day after the last bleed date of the final panel member.
[5] No panel members were identified as reactive by Multiplo HBc/HIV/HCV. For the purpose of calculations, 29 days was used for the Multiplo HBc/HIV/HCV detection and 12 days was used as the difference in days to HIV antibody detection, this represents one day after the last bleed date of the final panel member.

Conclusions

Multiplo HBc/HIV/HCV POC enables healthcare providers to maximize available resources, by simultaneously providing rapid results for three diseases with one drop of blood and one test device. Such an approach is beneficial to populations at risk for these infections whose access to care may be limited and who may be easily lost to follow-up, especially as these results show simultaneous detection can be achieved without compromising performance during early infection. Multicenter trials to generate additional performance data are underway in the USA.

medmira
helping people know...®