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Evaluation of the Roche Cobas TaqScreen Multiplex V 2.0 Test for Blood Screening: A Saudi Arabian Study

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Abstract: Clinical specificity and genotype/subtype detection of viruses using the Cobas TaqScreen MPX system V 2.0, which is a nucleic acid test (NAT) that uses multiples for hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) strain variants, were investigated. The 95% LOD was employed in the experiment and the results returned a positive accuracy of 99.6%. which is consistent with the expected success rate as specified by Roche.

Keywords: NAT, Multiplex, PCR

Introduction

Cobas TaqScreen MPX test V 2.0 is a real time, multiplex PCR test used in detecting the presence of human immune-deficiency virus type 1(HIV-1), groups O and M, human immunodeficiency virus-2 (HIV-2), HCV hepatitis C virus (HCV), and hepatitis V virus (HBV) in the plasma from organ, blood and tissue donors (Jarvis *et al*., 1853-1861). The MPX V 2.0 test simultaneously tests for the mentioned viruses in one sample, saving time and increasing efficiency. This is particularly important in field situations or emergencies where there is a need for quick and accurate screening of blood donations. The test also allows for two separate channel signal detections facilitating the simultaneously monitoring of viral targets and also allows complete process internal control throughout real time PCR; hence this enhances the integrity of the results (Schmidt *et al.,* 37-46). With the MPX V 2.0 test, real-time PCR does not require any calibration or stabilised reagents; this further reduces costs and saves time. Nucleic Acid Tests (NAT) for blood, tissue and organ donations for the HCV ribonucleic acid (RNA), HIV RNA and HBV deoxyribonucleic acid (DNA) are mandated tests required for every donor, and are performed by testing the plasma for every donated organ, tissue, or blood (Saldanha).

The MPX V 2.0 test that uses the Cobas s 201 system is intended for qualitative invitro tests for directly detecting the HIV-1 virus with group O RNA, HIV-2 RNA, HBV DNA, and HCV RNA in the plasma of humans. It is intended for screening the donor blood samples, organs and tissues to ensure that transfusions and organ transplants are safe for the recipients. The test can also be used on specific organs obtained from cadavers (donors whose hearts are not beating), and on organs obtained from donors whose hearts are still pumping. The test, however, is not intended for use in cord blood samples, but for use in combination with licensed HIV, HBV and HCV serology tests ('Roche Canada'). While the MPX V 2.0 test can be used in detecting HIV-2 RNA and HIV-1 RNA (group O), the detection of plasma specimens or anti HIV-2 antibodies or HIV-1 antibodies for group O in plasma specimens is not yet possible. Moreover, although the test kit has been approved and certified as effective for testing the presence of HBV, HIV and HCV viruses, it is imperative that the manufacturer's claims are verified through independent the testing of the MPX V 2.0 kit. In this study, various samples were tested using the kit, and the results analysed and evaluated to determine its effectiveness. Hypothesis

The MPX V 2.0 test, carried out the Cobas 201 system, is more than 95% effective in detecting viral infections in organs, blood, or tissues for the presence of HIV-1 and HIV- 2, HCV and HBV

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Literature review

The detection of HBV, HCV and HIV is essential in controlling the spread of these diseases to individuals who are not infected. The viruses have an extremely high rate of replication, and also being undetectable using serological markers; it is therefore important that effective mechanism be used for detection (American Association of Orthopedic Surgeons, Wilson, 138). This is particularly so where transmission and infection of uninfected individuals can be mediated by medical means. Transfusions of blood and tissue or organ transplants usually happen in emergencies; a fast means of accurately screening the blood for such viruses is of absolute essence. MPX V 2.0 directly tests for the presence of the three viruses' genetic components rather than for antigens or antibodies. This makes it a direct way of determining the presence of any of the three viruses and is especially useful in detecting early infections of the three virus classes earlier than antigen or antibody detection systems. The system has been proven to be 96 % accurate in detecting the presence of the three viruses. However, false positive results are possible in the event that cross-specimen contamination occurs during the handling or processing of the specimens (Health Canada). False positives may also occur in the Cobas MPX V 2.0 test if cross reactivity occurs with assay cellular components. However, the process has a very high level of sensitivity such that any positives will not be missed (Labmedica).

Method

The Cobas TaqScreen MPX V 2.0 was tested for clinical specificity, clinical sensitivities, genotype/ subtype, analytical sensitivities, and reproducibility for the tests' coverage. The evaluation for analytical sensitivity was done for the sensitivity levels of the Cobas MPX for HBV, HCV, and HIV using 5000 donor samples- the testing used a 95% limit of detection (LOD) for the MPX Test.

Materials

The Cobas MPX test was undertaken by means of the Cobas s 201 platform. The s 201 platform is composed of an automated blood sample pooling system in which blood donations are tested, using the Hamilton star IVD Pipettor. Samples are automatically prepared via the CAP (Cobas AmpliPrep) instrument and the automated amplified real time PCR. Detection occurs through the CTM (Cobas TaqMan) analyzer. The testing occurs through the nucleic acid amplification process to detect the presence HIV, HBV and HCV in the samples (Cabuang and Vinicci).

Results

The results of the tests are shown in Appendix I

All the samples that were tested showed reactions with the Cobas MPX V 2.0 test on the 201 s platform. Sample 1 was reactive for the HBV test at a CT value of 36%. Some HBV tests were positive, with 4 turning out as being negative, despite showing a reaction. The same trend was observed for the HIV and the HCV tests. The observed results were largely as expected, except for eight instances in which the MPX test showed a reaction in a negative sample test. The negative samples that reported a reaction are listed below along with the test and the respective CT values

Number	Result	CT Value %	NAT
4	Reactive	36	HBV
6	Reactive	37	HCV
7	Reactive	28	HCV
16	Reactive	34	HBV
17	Reactive	18	HBV
31	Reactive	36	HBV
32	Reactive	24	HCV
39	Reactive	35	HBV

Most of the negative results in which a reaction took place are HBV (62.5%) viral tests, with the remaining being HCV tests (37.5%). The negative HBV results had CT values of 36, 34, 18, 36 and 35 possibly because of the latency or window period before the virus causes the body to develop antibodies. The positive HBV results had a CT range between 26 and 35. The negative HCV results in which a reaction was reported had CT values of 37, 28 and 24, while the positive results had CT values ranging from 18 to 37. However, all the HIV test results were positive and were as expected. The negative results for the HBV tests and HCV tests can be regarded to be because of the viral window period as there was a positive NAT reaction although the serology results were negative for the presence of a HBV or HCV virus.

Discussion

Infections transmitted through the transfusion of blood, organs, and tissues are a present and ongoing challenge for the medical and transfusion fields. During blood, organ, or tissue transfusions, there is almost always an emergency because lives may

depend on the transfusion. It is therefore important that the donor organs, tissues, or blood are thoroughly and quickly screened for the presence of the deadly HIV, HCV and HBV (Peterson 83). The screening can be effectively done using the Cobas TaqScreen MPX V 2.0 test on the Cobas s 201 system. The results show that there were eight negative serology results for the HBV and HCV although there was a positive NAT result. The MPX V2.0 test is designed to test for the presence of viruses during the pre sero-conversion window period when a viral infection has not resulted in the production of antibodies by the body's immune system or antigen variants of the viruses. The negative serology tests showed a reaction at varying CT levels, but no virus and no antibody or antigen reactions although there was a viral presence detected because of the positive NAT reactions. All the negative serology results were either for the HBV or the HCV tests. The HBV test using the Cobas TaqScreen MPX system was very highly sensitive in detecting the viruses and therefore is an accurate and effective method for viral infection detection in tissues, blood or organs for the HBV tests, followed by the HCV test (in decreasing order of accuracy, respectively. The results of the experiment show 99.9% accuracy in the detection of the HIV-1 and HIV-2, HCV and HBV. The test kit showed the highest sensitivity for the HIV-1 and HIV-2 tests, with both strains being positively identified. The negative serology results (false positives) observed in the HBV and HCV strains are because of the pre-sero-conversion viral infection window period before antibodies or antigen viral variants develop. The results show that the accuracy of the Cobas TaqScreen MPX system is below the officially published level of 95-96 % range.

Statistical analyses

The results were analysed for standard deviation using SPSS with the following results computed

Sample Standard Deviation, s:	6.29
Sample Standard Variance, s^2	39.57
Total Numbers, N	47.00
Sum:	1314.10
Mean (Average):	27.96
Population Standard Deviation, σ	6.22
Population Standard Variance, σ^2	38.72
T value	30.077
DF	46
P value at 95% confidence levels	0.0001

The results show that the evidence on the accuracy of the MPX v2.0 test system is very strong at 95% confidence levels as shown by the t values and the p value. This means that the hypothesis is accepted as true.

Conclusions

The Cobas TaqScreen MPX Test is a real time, multiplex PCR test that is used in detecting the presence of HIV-1 groups O and M, HIV-2, HCV and HBV in the plasma from organ, tissue, and blood donors. The Cobas TaqScreen MPXV 2.0 test simultaneously tests for the presence of HBV, HCV and HIV in one sample, saving time and increasing efficiency. It is an accurate mechanism for positively detecting the presence of the HBV, HCV and HIV in plasma samples. This makes it a suitable method for rapid and accurate results. The test kit has an official accuracy of 95% in detecting HBV, HCV, and HIV. The experimental results yielded a 99.9% level of accuracy out of the 50 samples tested with the Cobas TaqScreen MPX v 2.0 systems on Cobas s 201. The negative serology results in which reactions were reported are because of the viral window period before sero-conversion to produce antibodies or antigenic variants. The mean 95% upper and lower limit levels for the LOD for testing for HIV (1 and 2), HCV and HBV were 99.9, 98.9 and 99.2 respectively. There were 50 samples representing 5000 donors used in the experiment. All the samples gave predictive and expected results of accurate detection, except in eight samples that gave negative serology results despite showing a positive NAT reaction. The overall accuracy of the Cobas TaqScreen MPX system was 99.9%, which is higher than the earlier stated accuracy of 95% that is the officially published level. The test method and platform were confirmed as being highly sensitive, flexible, specific, and robust. The MPX v2.0 is therefore an accurate test in ensuring safe medical interventions involving transfusions and transplants and can detect the HIV, HCV and HBV infections much before they produce antibodies or antigenic variants. The research recommends further investigations to determine the days the MPX v2.0 can detect HBV infection before the HBsAg can be detected in enzyme immune-assays. This should also be done for the number of days the MPX v2.0 can detect the HCV RNA before the HCV antibodies are produced and the days MPX v2.0 can detect HIV before HIV RNA and antibodies can be detected- The hypothesis is accepted as true based on the t value and p value statistical analyses that show strong evidence of the systems accuracy and reliability.

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Appendix I: Results

NO.	DATE	SAMPLE NO.	RESULTS	CT value %	NAT	SEROLOGY RESULTS			
1	1/13/2014	A-14-000216	Reactive	36	HBV	HBs Ag	HBc Ab		
2	1/21/2014	A-14-000617	Reactive	25	HIV				HIV Ag+Ab
3	1/22/2014	A-14-000642	Reactive	9	HBV	HBs Ag	HBc Ab		
4	1/23/2014	A-14-000666	Reactive	36	HBV	NEGATIVE			
5	1/22/2014	A-14-000683	Reactive	19	HCV		HBc Ab	HCV Ab	
6	1/23/2014	A-14-000686	Reactive	37	HCV	NEGATIVE			
7	1/26/2014	A-14-000802	Reactive	28	HCV	NEGATIVE			
8	1/28/2014	A-14-000900	Reactive	27	HBV	HBs Ag	HBc Ab		
9	2/2/2014	A-14-001016	Reactive	31	HBV	HBs Ag	HBc Ab		
10	2/3/2014	A-14-001045	Reactive	28	HBV	HBs Ag	HBc Ab		
11	2/5/2014	A-14-001139	Reactive		HBV	HBs Ag	HBc Ab		
12	2/11/2014	A-14-001313	Reactive	20	HCV		HBc Ab	HCV Ab	
13	2/13/2014	A-14-001402	Reactive	29	HBV	HBs Ag	HBc Ab		
14	2/17/2014	A-14-001490	Reactive			HBs Ag	HBc Ab		
15	2/18/2014	A-14-001513	Reactive			HBs Ag	HBc Ab		
16	4/2/2014	A-14-001663	Reactive	34	HBV	NEGATIVE			
17	4/2/2014	A-14-001671	Reactive	18	HBV	NEGATIVE			
18	2/25/2014	A-14-001748	Reactive	34.3	HBV	HBs Ag	HBc Ab		
19	2/26/2014	A-14-001790	Reactive	25	HBV	HBs Ag	HBc Ab		
20	2/26/2014	A-14-001849	Reactive	29	HBV	HBs Ag	HBc Ab		
21	3/3/2014	A-14-001981	Reactive	29.2	HBV	HBs Ag	HBc Ab		

22	3/12/2014	A-14-002339	Reactive	34	HBV	HBs Ag	HBc Ab		
23	3/13/2014	A-14-002370	Reactive	19	HCV			HCV Ab	
24	3/19/2014	A-14-002534	Reactive	35	HBV	HBs Ag	HBc Ab		
25	3/19/2014	A-14-002557	Reactive	29	HBV	HBs Ag	HBc Ab		
26	3/24/2014	A-14-002679	Reactive	26	HBV	HBs Ag	HBc Ab		
27	3/25/2014	A-14-002772	Reactive	34	HBV		HBc Ab		
28	3/25/2014	A-14-002773	Reactive	26	HBV	HBs Ag	HBc Ab		
29	3/31/2014	A-14-002942	Reactive	32	HBV	HBs Ag	HBc Ab		
30	4/1/2014	A-14-003018	Reactive	32	HBV	HBs Ag	HBc Ab		
31	4/8/2014	A-14-003158	Reactive	36	HBV	NEGATIVE			
32	4/9/2014	A-14-003290	Reactive	24	HCV	NEGATIVE			
33	4/14/2014	A-14-003431	Reactive	37	HBV		HBc Ab		
34	4/17/2014	A-14-003559	Reactive	24	HBV	HBs Ag	HBc Ab		
35	4/17/2014	A-14-003592	Reactive	33	HBV	HBs Ag	HBc Ab		
36	4/21/2014	A-14-003645	Reactive	18.7	HCV		HBc Ab	HCV Ab	
37	4/28/2014	A-14-003958	Reactive	38	HBV		HBc Ab		
38	5/13/2014	A-14-004121	Reactive	22	HBV	HBs Ag	HBc Ab		
39	5/13/2014	A-14-004335	Reactive	35	HBV	NEGATIVE			
40	5/14/2014	A-14-004570	Reactive	18	HCV		HBc Ab	HCV Ab	
41	5/15/2014	A-14-004582	Reactive	29	HBV	HBs Ag	HBc Ab		
42	5/15/2014	A-14-004609	Reactive	28	HBV	HBs Ag	HBc Ab		
43	5/15/2014	A-14-004630	Reactive	28	HBV	HBs Ag	HBc Ab		
44	5/20/2014	A-14-004730	Reactive	31.3	HBV	HBs Ag	HBc Ab		
45	5/20/2014	A-14-004748	Reactive	24.6	HBV	HBs Ag	HBc Ab		
46	5/22/2014	A-14-004844	Reactive	26	HBV	HBs Ag	HBc Ab		
47	5/22/2014	A-14-004844	Reactive	26	HBV	HBs Ag	HBc Ab		
48	5/25/2014	A-14-004937	Reactive	23	HBV	HBs Ag	HBc Ab		
49	5/25/2014	A-14-004943	Reactive	28	HBV	HBs Ag	HBc Ab		
50	4/8/2014	C-14-000190	Reactive	23	HCV			HCV Ab	
51	4/10/2014	A-14-00966	Reactive						HIV Ag+Ab