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IJCV INTERNATIONAL JOURNAL OF CLINICAL VIROLOGY
ISSN 2692-4994

Research Article

Mass Serological Screening in the Armed Forces Using the Serum-Pooling Method. Analytical Evaluation of the Chemiluminescence Method

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Submitted: February 06, 2025
Approved: February 17, 2025
Published: February 18, 2025

How to cite this article: Abi R, Ameur O, Hassine S, Chanhih N, Ouannass S, Goura H, et al. Mass Serological Screening in the Armed Forces Using the Serum-Pooling Method. Analytical Evaluation of the Chemiluminescence Method. Int J Clin Virol. 2025; 9(1): 001-004. Available from: https://dx.doi.org/10.29328/journal.ijcv.1001062

Keywords: Screening; Pooling; Chemiluminescence

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Abstract

Mass serological screening in the Armed Forces involves detecting serological markers of chronic infections, particularly viral hepatitis B and C, syphilis, and HIV among young military recruits. The objective of this study is to evaluate the analytical performance of the chemiluminescence technique (CMIA-Architect i2000 SR) in mass serological screening using the serum-pooling method at the virology laboratory of the Mohammed V Military Teaching Hospital.

Samples with known serological results (positive/negative) were grouped into pools of different sizes (2, 5, 10, and 15 sera). These pools were tested using chemiluminescence (CMIA-Architect i2000 SR). A cost analysis was conducted to assess potential savings based on seroprevalence and pool size.

Results showed that the pooling method maintained 100% specificity. Overall sensitivities for detecting positive samples were 93.1% for HBV, 83.33% for HCV, and 86.36% for HIV. Positive and negative predictive values were high for all three viral markers, highlighting the reliability of the pooling method. Additionally, this approach generated significant cost savings, ranging from 46% to 80%.

Conclusion: This study demonstrated the solid analytical performance of the chemiluminescence technique (CMIA-Architect i 2000 SR) using the serum-pooling method for detecting HBV, HCV, and HIV serological markers in low-seroprevalence regions.

Introduction

The concept of sample pooling is emerging as a promising strategy to address the logistical and economic challenges associated with large-scale viral testing [1]. Instead of subjecting each individual sample to a separate test, pooling allows multiple samples to be combined into a single composite pool, and only those from positive pools can be tested individually. This approach has the potential to reduce testing costs, minimize reagent consumption, optimize laboratory staff organization, and accelerate the testing process [1].

Sample pooling is not a new approach, it has been successfully used in various infectious disease cases in the past [2]. The history of pooling dates back several decades, when Dorfman proposed and applied the pooling method to screen soldiers for syphilis during World War II [2]. More recently, the global COVID-19 pandemic has re-emphasized the importance of large-scale testing while highlighting the limitations of single-sample testing approaches [1].

The objective of this work is to evaluate the analytical performance of the Chemiluminescence Microparticle Immunoassay (CMIA) technique when applied to pooled samples, compared to the individual samples. The study will focus on detecting serological markers of the Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and Human Immunodeficiency Virus Infections (HIV). The second objective is to determine whether this approach provides significant cost savings while maintaining satisfactory test performance.

Materials and methods

The study was conducted in the virology laboratory of the

Mohammed V Military Teaching Hospital in Rabat over a four-month period, from September to December 2024. Samples were collected in dry tubes along with an information sheet specifying the patient's age, sex, and clinical details. The detection of serological markers for HBV (HBsAg), HCV (anti-HCV antibodies) and HIV (p24 antigen and anti-HIV1+2 antibodies) was performed using the chemiluminescence technique (CMIA-Architect i2000 SR) in both unitary (single sample) mode and using serum pooling method.

Sera that had already tested positive for the three markers at known titers were pooled into groups of 2, 5, 10, and 15 sera. Each pool contained one positive sample, with the remaining sera being negative for the markers in question. Simultaneously, a similar approach was used to verify whether negative samples maintained their negative status when combined into pools.

Pools were prepared by pipetting 100 µl of each serum. The number of samples testing positive and negative for the studied serological markers is summarized in Table 1.

Results

For Hepatitis B Virus (HBV)

None of the pools composed of sera negative for HBsAg gave a positive result, indicating 100% specificity at the pool level. On the other hand, among 29 positive samples, 27 sera tested positive, however, two weakly positive samples (titers: 1.33, 2.65 IU/mL) resulted in false-negative pools. This resulted in an overall sensitivity of 93.1% in the tested population. No false-positive results were detected during these experiments. The positive predictive value (PPV) and negative predictive value (NPV) were 100% and 86.66%, respectively (Table 2).

Concerning Hepatitis C Virus

None of the pools composed of sera negative for anti-HCV antibodies (anti-HCV Ab) generated a positive result, confirming 100% specificity at the pool level. Additionally, out of 24 anti-HCV Ab-positive samples, 20 sera gave positive results when tested as pools. However, the four remaining pools, containing a positive serum each, with Upper Reference Limit (URL) titers of 1.2, 1.42, 4.61, and 6.97, tested negative. This resulted in an overall sensitivity of 83.33% in the tested population. The PPV and NPV were 100% and 71.42%, respectively (Table 3).

For Human Immunodeficiency Virus

None of the pools consisting of sera negative for HIV has produced a positive result, confirming a 100% specificity at the pool level. Furthermore, among 22 samples, positive for anti-HIV, 19 sera gave positive results when tested in pools. However, the three remaining pools, each containing one positive serum with URL titers of 1.13, 3.5, and 5.56, tested negative. This resulted in an overall sensitivity of 86.36% in the tested population. The PPV and NPV were 100% and 76.92%, respectively (Table 4).

Discussion

Hepatitis B Virus serological screening

The results obtained revealed complete consistency in performance across all pool sizes. A specificity of 100% was observed, meaning that pools composed of negative samples did not reveal any false-positive results. The overall sensitivity for detecting HBsAg-positive samples was 93,1%, with two pools containing weakly positive samples (whose URL titers are 1.33 and 2.65) testing negative. This could be attributed to the increased dilution of the antigen in the pools. The positive predictive value (PPV) and Negative Predictive Value (NPV) for HBV were 100% and 86.66%, respectively, underscoring the reliability of the pooling technique.

These findings align with previous studies that also reported high specificity and sensitivity for the pooling technique in HBV detection [3]. A study conducted on blood donors from two separate sites evaluated the performance of

Table 1: Number of samples tested using the pooling method for each virus (HBV, HCV and HIV).

	VHB	VHC	VIH	
Négative	13	10	10	Total of samples
Positive	29	24	22	
Total	42	34	32	108

Table 2: Analytical performance of the chemiluminescence method (CMIA-Architect i 2000 SR) using the pooling method for serological screening of HBV.

		Unit sample results		
		Positive	Negative	
Pool results	Positive	29	13	
		True Positive 27	False Positive 0	PPV 100%
	Negative	False Negative 2	True Negative 13	NPV 86.66%
Sensitivity = 93.1%; Specificity = 100%				

Table 3: Analytical performance of the chemiluminescence method (CMIA-Architect i 2000 SR) using the pooling method for serological screening of HCV.

		Unit sample results		
		Positive	Negative	
Pool results	Positive	24	10	
		True Positive 20	False Positive 0	PPV 100%
	Negative	False Negative 4	True Negative 10	NPV 71,42%
Sensitivity = 83,33% Specificity = 100%				

Table 4: Analytical performance of the chemiluminescence method (CMIA-Architect i 2000 SR) using the pooling method for serological screening of HIV.

		Unit sample results		
		Positive	Negative	
Pool results		22	10	
	Positive	True Positive 19	False Positive 0	PPV 100%
	Negative	False Negative 3	True Negative 10	NPV 76,92%
Sensitivity = 86,36% Specificity = 100%				

an HBsAg enzyme immunoassay on pools of 3 to 24 samples and compared it to individual testing. Both methods 'sensitivity and specificity were consistent with manufacturer-reported values of 93% to 99% [4].

The negative predictive value, representing the probability that a negative test result corresponds to the absence of disease, was estimated at 86.66%. This value is explained by the two pools which were identified as non-reactive (negative), each one contains a weakly positive serum, whose URL values are 1.33 and 2.65. This finding suggests that weakly positive samples may undergo increased dilution in pools, resulting in false negatives. Similar findings were reported in a previous study evaluating HBV DNA detection in pooled samples, which found a specificity and PPV of 100%, but a sensitivity and NPV of 66.7% and 96.4%, respectively. The reduced sensitivity was attributed to the inability to detect nine samples with low HBV DNA levels (< 15 IU/mL) [5].

Furthermore, the previously cited study (4) provided an explanation for the rare cases of false negative results (4), suggesting that this could be due to the presence of anti-HBs antibodies, which neutralize HBsAg and lead to low or negative HBsAg detection. This is particularly relevant in regions with high anti-HB prevalence due to natural infections or mass vaccination programs. However, according to a separate study [4]; HBV antigen-antibody interactions were more pronounced in pools left overnight compared to those tested immediately. To minimize this effect, the authors recommended performing HBsAg testing on the same day as the pooling to reduce potential interference with anti-HBs antibodies that may be present in the pooled samples.

Hepatitis C Virus serological screening

The evaluation of the analytical performance of the chemiluminescence method using the pooling technique for HCV serological screening also produced significant results. The overall sensitivity was 83.33%, slightly lower compared to HBV, with four weakly positive pools (whose URL titers are 1.2, 1.42, 4.61, and 6.97) identified as non-reactive. This reinforces the fact that weakly positive samples are more likely to give false-negatives results due to dilution. The PPV and NPV for HCV were 100% and 71.42%, respectively.

These results are consistent with previous studies that demonstrated the high specificity and sensitivity of the pooling technique. One study evaluated the feasibility and performed a cost/benefit analysis of a pooling protocol for enzyme immunoassays to detect anti-HCV antibodies. The results showed a sensitivity of 100%, a specificity of 99.2%, a false positive rate of 0.8%, and a false negative rate of 0%. The cost-benefit analysis revealed a 69.3% reduction in costs, leading the authors to recommend pooling for large-scale screening in low-risk populations with low seroprevalence [6].

Unlike HBV, false-negative results for HCV do not appear to be attributed to antigen-antibody reactions. According to one study, anti-HCV antibodies were detectable up to a dilution of 1/80 [7]. This was confirmed by other studies, which found no evidence of neutralization between HCV antigens and antibodies and that the antigen-antibody complexes did not block the immunological reaction, ensuring the detection of antibodies [6].

Human immunodeficiency virus serological screening

The results were similar to those obtained for HBV and HCV, with specificity maintained at 100% across all pools tested, and no false-positive results detected. Meanwhile, the overall sensitivity was 86.36%, slightly higher than that for HCV with three weakly positive samples (whose URL titers are 1.13, 3.5, and 5.56) tested negative in the respective pools, highlighting a potential reduction in sensitivity for weakly positive samples. The PPV and NPV for HIV were 100% and 76.92%, respectively.

A study comparing individual testing to pooled testing (with pools of 5 and 10 samples) found no loss of sensitivity or specificity for pools of 5 samples. However, pools with 10 samples showed reduced sensitivity for samples with low antibody titers. Additionally, an economic analysis revealed that pooling could reduce screening costs by 70% and shorten analysis times. The authors concluded that pooling five samples for HIV testing can indeed result in substantial savings. However, in countries with higher HIV prevalence (2% - 3%), savings may be lower [8].

The NPV was estimated in our study at 76.92%. This implies that, in approximately 23.08% of cases, the technique may falsely indicate that a person is negative when they are actually HIV positive. This was attributed to low viral titers in weakly positive samples, which were diluted during pooling, reducing their detectability. Other studies have suggested that false negative results may also be attributed to complex interactions between viral antigens and HIV antibodies, which can interfere with test reactivity [9]. Therefore, in order to optimize the reliability of pooling tests, it is recommended that they be used primarily in low-prevalence populations [10].

Limitations and recommendations

We have demonstrated that the CMIA method applied to pooled sera is a sensitive and specific technique for detecting HBsAg, HCV antibodies, and HIV antibodies in a low-prevalence region. This performance was maintained even in pools containing up to 15 samples. However, the effectiveness of the pooling technique may vary depending on local seroprevalence and marker titers. Weakly positive samples can affect the sensitivity of the method. The potential neutralization of positive samples by antibodies, particularly in HBsAg detection, must be considered. Finally, although our

study showed that analytical performance was independent of pool size, different contexts may yield different outcomes, as pool size can affect the method's effectiveness.

Conclusion

This study confirmed the robust analytical performance of the chemiluminescence method for serological screening of HBV, HCV, and HIV using the pooling technique, highlighting its significant potential for cost savings. However, the sensitivity and specificity of the method can vary depending on several factors, including marker titers and local seroprevalence. Therefore, the selection of the appropriate pool size is essential to optimize the effectiveness of this approach. Further studies are needed to confirm our findings.

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