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Validation of Specimen Pooling Versus Individual Samples for Screening of Viral Markers and Syphilis in Blood Bag Strategy Single-Center Study

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ABSTRACT

Background: Blood safety is a crucial health concern, and it is still a problem on a worldwide scale. Pool-testing approach is to combine multiple samples and test them as one pool. A pool-testing strategy can shorten the screening time and increase the test rate, especially during times of inadequate reporting speed and limited test availability.

Aim: This research aimed to assess the effectiveness of the pool-testing strategy in Egyptian blood banks and to develop a pooling test to be used in the routine screening of blood donors. We suggest that the use of pooling for blood screening is beneficial, especially in our country, which has a high rate of hepatitis infection and recent extensive government control measures, as well as the importance of potential cost savings in Egypt.

Subjects and methods: Sera from 619 volunteer blood donors approaching Suez Canal University Hospital's Blood Transfusion Department, were screened individually for Syphilis and viral infectious markers HBV, HCV, and HIV by ELISA, then pooled into pools of five and retested.

Results: The results showed sensitivity, specificity, and accuracy of syphilis and HBV tests were 100% on pooled samples while HCV test showed sensitivity 71%, specificity 100%, and accuracy 99.68%.

Conclusion: Our present study demonstrated the importance of a pooling protocol and that a pooling scheme of five samples is helpful for mass screening for syphilis and viral markers in subjects from large populations as blood donors without loss of accuracy.

Keywords: Syphilis- HBV- HBV- HIV- Pooling- Screening- Transfusion.

INTRODUCTION

For decades, it has been known that transfusion of blood products and plasma derivatives can transmit viral infections, even though it has to be an inevitable result of a life-saving procedure. A viral infection can potentially be transmitted by transfusion when it has a long or brief asymptomatic blood-borne stage, as well as having the ability to remain in collected blood products and causing infection intravenously [(1)]. The potential of viral transfusion-transmitted infections (TTI) has consistently decreased during the previous forty years with the development of laboratory investigations for hepatitis B virus (HBV) surface antigen (HBsAg) in the 1970s [(2)]. Alanine aminotransferase (ALT) and HBV core (anti-HBc) antibodies testing started in the 1980s to decrease the spread of non-A non-B viral hepatitis. Then, the emergence of the human immunodeficiency virus (HIV) and the subsequent rapid identification of hepatitis C virus (HCV) resulted in extraordinary attempts to stop the spread of these new blood-borne viruses and the introduction of antibody tests for both of these agents [(3)].

To rule out those who are susceptible to transmit viral infection, a donor screening procedure has been established and implemented. This procedure involves the donor's medical history and a short physical examination. The following critical step in lowering viral TTI was accomplished with the establishment of serologic testing [(4)]. Blood banks are under increasing economic pressure regarding the addition of any costly molecular testings for blood donations screening for any newly (re)-emerging viruses. In addition to the increased running expenses for instruments and reagents, as well as the requirement for cold-chain transport and reagents storage [(5)]. So, decreased cost-effectiveness encouraged the advent of a number of cost-cutting strategies. The enormous funding to gain trust in the blood supply safety has led to a distinct issue: the discrepancy that the health care yield is usually extremely poor, and the resources could be more effectively used elsewhere [(6).]

Blood safety at all costs comes with risks that might affect sustainability and ability to face new problems [(7)]. The technique of sample pooling was demonstrated to be practical for extensive sero-epidemiological research for years ago [(8)]. This technique utilizes enzyme-linked immunosorbent assays (ELISAs) to screen samples from multiple demographic groups, which minimizes resources in terms of time, work, and supplies [(9)].

Transfusion safety continues to be the main driver for the invention of technically advanced technologies that can be applied for blood screening for facing the ongoing (re)emergence of novel viruses and variations of existing viruses [(10)]. Our research was planned to assess the detection of Syphilis and viral infectious markers HBV, HCV, and HIV by using ELISA in plasma pools in blood donors rather than in individual samples to lower the time of screening and cost burden on blood banks in Egypt as a developing country.

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SUBJECTS AND METHODS

The study was carried out in blood transfusion center at the Hospital of Suez Canal University in the time from January 2022 to July 2022. All participants were over 18 years of age, had given informed consent, and attended blood banks for donation.

Demographic data were obtained from donors after the preliminary interview and clinical examination. All blood donations were subjected to the necessary routine screening tests at blood banks' serological labs (blood group, cross-matching, Syphilis antibodies, anti-HCV antibodies, HBs Antigen, and HIV antibodies) following the Egyptian national standards for blood transfusion.

A total of 619 samples were gathered from voluntary donors then centrifuged at 10,000 rpm for 10 minutes. Plasma was frozen 6 hours of collection at −80°C until it underwent testing. Samples were initially tested as single samples by ELISA for Syphilis (Syphilis Total Ab, Bio-Rad, France), HIV (Genscreen^{TM} ULTRA HIV Ag-Ab, Bio-Rad, France), HBV (Monolisa^{TM} HBs Ag ULTRA, Bio-Rad, France), and HCV (Monolisa^{TM} HCV Ag-Ab ULTRA V2, Bio-Rad, France). Then, each five samples were grouped to make a pool. A total of 124 pools were prepared. All pools were retested, and individual sample testing was performed on all members of a positive pool.

Statistical analysis of the data:

Data were entered to the computer and processed by version of IBM SPSS 25.0 software programme. (Armonk, NY: IBM Corp). Categorical data were shown as numbers and percentages. The Chi-square test was used to compare any two groups. But when more than 20% of the cells have an estimated count below 5, Fisher Exact correction test was used instead. To express quantitative data, a range, mean, standard deviation, and median were used. Intra class Correlation coefficient was used for the agreement between individual testing and pooling. The ICCs were classified using a system suggested by Koo and Li [11] as follows: <0.50 poor reliability; 0.50 to <0.75 moderate reliability; 0.75 – 0.90 good reliability; > 0.90 excellent reliability. The Kappa test was used for agreement between individual testing and pooling. The Kappa was categorized as: ≤ 0.20 poor agreement; 0.21 – 0.40 fair agreement; 0.41 – 0.60 moderate agreement; 0.61– 0.80 good agreement; > 0.80 very good agreement. The significance of the obtained results was assessed at the 5% level.

Ethical approval:

All patients gave written consents to take part in the current research. They were notified about the research, purpose, and its benefits. Consents included permission of the patients for using the data in their files from Suez Canal University Hospital in our research. To ensure data confidentiality a code number for linking the data from each subject was used. All data obtained from everyone were strictly confidential and were not used outside this study. The research was conducted with approval from The Scientific Research Ethical Committee of Suez Canal University. This work was carried out in line with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for human studies.

RESULTS

Initially, individual samples were screened and then in pools of five sera. Positive pools were identified, and all its samples were retested by individual screening. Table (1) showed that the absorbance (OD) of five-sample pools was lower than those found in the individual screening.

Table (1): Distribution of the OD values in each parameter (n = 619)

	Individual	Pooling
Syphilis		
Mean ± SD.	0.032 ± 0.084	0.031 ± 0.083
Median (Min. – Max.)	0.023 (0.002 – 2.050)	0.021 (0.010 – 2.0)
HIV		
Mean ± SD.	0.091 ± 0.073	0.051 ± 0.034
Median (Min. – Max.)	0.051 (0.017 – 0.630)	0.048 (0.011 – 0.430)
HCV Abs		
Mean ± SD.	0.132 ± 0.949	0.051 ± 0.149
Median (Min. – Max.)	0.036 (0.010 – 20.20)	0.032 (0.001 – 2.210)
HBs Ag		
Mean ± SD.	0.149 ± 0.226	0.039 ± 0.119
Median (Min. – Max.)	0.037 (0.004 – 3.348)	0.028 (0.001 – 2.235)

SD: Standard Deviation, **OD:** optical density

Syphilis test was performed on 619 individual serum samples revealing one true positive sample. When individual tests results were used as the control, there was no false negative. Thus, the serum-pooling protocol's sensitivity, specificity, accuracy, and positive and negative predictive values were all 100% for syphilis. So, the prevalence of syphilis infection was 0.2%.

While, testing **HIV** on 619 individual serum samples did not reveal any positive samples. And none of the 124 serum pools were revealed to be positive. Thus, sensitivity, specificity, and accuracy of the serum-pooling protocol cannot be calculated. So, the prevalence of HIV infection was 0%.

In addition, testing HCV-Abs on pooled samples followed by testing individual samples, 612 of 619 serum samples revealed to be true negative with a true negativity rate and specificity 100%, and NPV 99.67%. Furthermore, five samples were revealed to be true positive giving a true positivity rate and sensitivity 71.4%, and PPV 100%. Whereas two of the samples were false negative with 28.6% false negativity rate.

Thus, the accuracy of the test was 99.68%. So, the prevalence of HCV infection was 0.8%. Meanwhile, **HBs Ag** was tested on pooled samples followed by testing individual samples, 3 of 619 serum samples revealed to be true positive. Thus, accuracy, and positive and negative predictive values of the serum-pooling protocol were 100 percent for HBV. So, the prevalence of HBV infection was 0.5% (Table 2).

Table (2): Sensitivity, specificity, and accuracy for Individual vs Pooling (n = 619)

Pooling	Individual		Sensitivity	Specificity	PPV	NPV	Accuracy
	Negative	Positive (>1)					
Syphilis	**(n = 618)**	**(n = 1)**					
Negative	618 (100%)	0 (0%)	100	100	100	100	100
Positive (>1)	0 (0%)	1 (100%)					
HIV	**(n = 619)**	**(n = 0)**					
Negative	619 (100%)	–	–	–	–	–	–
Positive (>1)	0 (0%)	–					
HCV Abs	**(n = 612)**	**(n = 7)**					
Negative	612 (100%)	2 (28.6%)	71.4	100	100	99.67	99.68
Positive (>1)	0 (0%)	5 (71.4%)					
HBs Ag	**(n = 616)**	**(n = 3)**					
Negative	616 (100%)	0 (0%)	100	100	100	100	100
Positive (>1)	0 (0%)	3 (100%)					

PPV: Positive predictive value **NPV:** Negative predictive value

According to kappa classification, results of Syphilis, HCV, and HBV tests on pooled samples showed very good agreement with individual ones (Table 3). While, the Intraclass correlation coefficient indicated that HIV and HCV tests showed poor reliability, HBV test showed moderate reliability and Syphilis test showed excellent reliability when performed on pooled samples (Table 4).

Table (3): Agreement between Individual and Pooling (n = 619)

Pooling	Individual		χ^2	p	Kappa	Percent of agreement
	Negative	Positive (>1)				
Syphilis	**(n = 618)**	**(n = 1)**				
Negative	618 (100%)	0 (0%)	619.0*	$^{FE}p=0.002^*$	1.0	Very good
Positive (>1)	0 (0%)	1 (100%)				
HIV	**(n = 619)**	**(n = 0)**				
Negative	619 (100%)	–	–	–	–	–
Positive (>1)	0 (0%)	–				
HCV Abs	**(n = 612)**	**(n = 7)**				
Negative	612 (100%)	2 (28.6%)	440.7*	$^{FE}p=<0.001^*$	0.832	Very good
Positive (>1)	0 (0%)	5 (71.4%)				
HBs Ag	**(n = 616)**	**(n = 3)**				
Negative	616 (100%)	0 (0%)	619.0*	$^{FE}p=<0.001^*$	1.0	Very good
Positive (>1)	0 (0%)	3 (100%)				

χ^2: **Chi-square test**, FE**: Fisher Exact,** κ: **kappa test,** * Statistically significant value $p \leq 0.05$, Kappa was classified as: ≤ 0.20 poor agreement; 0.21 – 0.40 fair agreement; 0.41 – 0.60 moderate agreement; 0.61 – 0.80 good agreement; > 0.80 very good agreement.

Table (4): Intraclass Correlation coefficient for different parameters (n = 619)

Individual vs Pooling	ICC coefficient	95% C.I	p
Syphilis	0.970	0.965 – 0.974	<0.001*
HIV	0.086	0.007 – 0.164	0.016*
HCV Abs	0.224	0.148 – 0.297	<0.001*
HBs Ag	0.546	0.488 – 0.599	<0.001*

CI: Confidence interval, LL: Lower limit, UL: Upper Limit, * Statistically significant value $p \leq 0.05$
ICCs were classified as follows: <0.50 poor reliability; 0.50 to <0.75 moderate reliability; 0.75 – 0.90 good reliability; >0.90 excellent reliability.

DISCUSSION

Around 92 million individuals worldwide donate blood each year [12]. Of these blood units, almost 1.6 million are wasted because they are contaminated with infectious pathogens [13]. In order to stop the transmission of these pathogens, collected blood units have to be serologically investigated, as advised by the World Health Organization (WHO) [14]. Despite all the followed guidelines, the risk of transmission of infections through the blood has always existed. This increases the issue of blood depletion and brought the attention to the need and the aim of guaranteeing global safe and available use of blood and its products [15]. Considering the high current market pricing of anti-HCV, Syphilis, HBs Ag, and HIV in Egypt, together with the personnel expenses, using the pooling technique might have saved millions of pounds annually.

The pooling technique we used is an adjustment of the traditional individual-serum testing used to screen huge scale of population for Syphilis, HBV, HIV, and HCV. So, the present work was done to examine the efficacy of pooling in blood donations to screen for Syphilis and viral infectious markers HBV, HCV, and HIV by using ELISA, to propose a pooling scheme with potentially accurate results.

A pooling technique for screening aids in enhancing clinical laboratories' diagnostic capabilities and providing access to broader viral hepatitis screening. Serological HCV testing is essential to avoid situations where RNA levels fluctuate [16]. Blood banks in Egypt rely mainly on serological assays for HCV screening [17]. In the current study, the prevalence of HCV infection was 0.8%. This low prevalence may be due to the nationwide strategy for HCV control, which is currently implemented, and the introduction of highly effective direct-acting antiviral medications, which are targeted to cure more than 250,000 infected people annually to reach a national prevalence of chronic HCV infection < 2% in 2025 [18]. Prior to this, Egypt was thought to have the greatest global HCV prevalence, with a 14.7% estimate in 2008 [19]. Later in 2015, a 10% estimate was provided [20]. Improvements in the prevention and screening of transmission should also be a priority in light of these actions.

In the USA, mini-pools of 16–24 samples have been implemented [21], even though 48–96 pools were used in other countries [22]. Using big pool sizes may be acceptable due to these countries' low HCV prevalence in comparison with Egypt. According to kappa classification, results of HCV tests on pooled samples in our study exhibited very good agreement with individual ones. The "pools of five" method for HCV screening in blood donors offers an affordable alternative to expensive diagnostic testing. When compared to using individual sera tests, an overall cost savings of more than 75% in labor work consumables and reagents is possible [19]. Additionally, pooling has been useful in generating prevalence rates that might be applied to set up screening guidelines [19].

Chronic viral hepatitis and its complications were listed as one of the top 20 causes of death according to the Global Burden of Diseases 2015 report [23]. HBV can cause acute, which can result in cirrhosis, fibrosis of the liver, and an increased risk of liver cancer. HBV infections affect 3.3 million people in Egypt [24]. In the current study, HBV test showed a very good percentage of agreement when performed on pooled samples. In 2015, Egypt Health Issues Survey (EHIS) conducted by the Egyptian Ministry of Health, estimated the rate of HBV infection in individuals aged 15–59 (15,777 samples) to be 1.4% with men having higher rates than women (1.9% versus 1.1%, respectively) [20]. Our study found that the prevalence of HBV infection was 0.5%. This is consistent with estimations from the WHO, which showed that HBV incidence among blood donors in high-, middle-, and low-income countries were 0.02%, 0.64%, and 3.59% respectively [25]. The seropositivity of HBsAg donors in Egypt declined from 1.1% in 2015 to 0.91% in 2018 [26]. The declining trend in HBV prevalence is most likely because of behavioral changes that reduce the spread of the infection.

One source of Syphilis transmission is blood transfusion [27]. Around 12 million people were infected with syphilis in 1999, with over 90% of cases in underdeveloped countries. Syphilis infections decreased in the developed world up to the 1980s and 1990s as a result of the extensive use of antibiotics. Rates of syphilis have been rising since the year 2000, this may be due to

unsafe sex. Additionally, it is estimated that T. pallidum infects 17.1 million persons between the ages of 15 and 49 each year [28]. Rapid plasma reagin (RPR) screening is used to both diagnose cases that have gone undiagnosed and to stop the spread of syphilis through blood and blood products [29]. The prevalence of syphilis was determined to be 0.2% in our study. This low syphilis seroprevalence found in the general population in Egypt may be a result of the country's Islamic religious laws. Similar results were found for Turkish blood donors (0.2% seroprevalence) [30] and Nigerian blood donors (0.2% seroprevalence) [31]. Our study's serum-pooling methodology had a 100% syphilis accuracy rate.

In 2019, there were roughly 1.7 million new HIV infections, which is the lowest estimate since 1990 and a 23% decline from the peak in 2010. But the worldwide goal of less than 500,000 new infections by 2020 has yet to be achieved [28]. In our study, the great news was that no cases of HIV amongst Egyptian donors and the prevalence of HIV infection was 0%.

These data suggest further research based on bigger populations; such research may serve to lessen the total burden on Egypt's already fragile healthcare system.

CONCLUSION

The pooling system for the detection of Syphilis and viral infectious markers is an effective and practical way to use in blood screening in Egyptian blood banks and countries having few resources. More nationwide research is required to assess its cost-effectiveness and the ongoing risk of contracting infections after getting a blood transfusion screened by serological pooling protocol.

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