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Comparison of human immunodeficiency virus and syphilis results in pooled sera versus individual samples of blood donors attending suez canal university hospital

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Abstract

Egypt is one of the countries where sexually transmitted diseases like human immunodeficiency virus (HIV) and syphilis are least prevalent. HIV and syphilis count less than one percent of total Egyptian population. An ELISA protocol for pooling serum samples is simple and may provide a way to reduce the cost and time needed for analysis. This study aimed to investigate the applicability and reliability of testing pooled sera of blood donors for HIV and syphilis compared to testing their individual sera and to assess the cost-effectiveness of this procedure. The study included 75 sera from randomly selected blood donors attending Suez Canal University hospital. Sera were screened by two ELISA kits, HIV Ag-Ab ELISA kit, and syphilis total antibody ELISA kit. Screening protocols were done by two sequential steps. At first, samples in pools of five were screened for both HIV and syphilis then, samples in positive pools were individually retested. There was no significant difference between the mean optical density for samples tested HIV and syphilis positive either individually or in pooled sera. There was no difference between the number of individual sera, tested positive for both HIV and syphilis and their pooled sera results (100 % positivity). There was significant decrease of the mean cost in one pool of 5 samples (16.5 L. E) in comparison to 5 individual samples (82.5 L. E) by HIV ELISA. Also, there was significant decrease of the mean cost in one pool of 5 samples (16 L.E) in comparison to 5 individual samples (80 L.E) by syphilis ELISA. In conclusion, the studied pooling protocol appeared reliable and can save up to 80 % of the cost for testing either HIV or syphilis by regular procedures.

Keywords: HIV, syphilis, pooling, ELISA, blood transfusion.

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Introduction

Egypt is one of the countries where sexually transmitted diseases like human immunodeficiency virus (HIV) and syphilis is least prevalent.[1] Egypt remains a low HIV prevalence country. According to the Joint United Nations Programme on HIV/AIDS (UNAIDS) 2016 statistics, there are about 11,000

people currently living with HIV in Egypt.[2] The blood transfusion services in Egypt began routine screening for HIV and syphilis on all blood donations since 1985. This practice was adopted because of the well documented risk of sexually transmitted infection associated with blood transfusion and resulted in an apparent low incidence of HIV and syphilis associated with blood transfusion.[1]

The cost for such screening program is substantial, and many developing countries, particularly in Africa where epidemics spread rapidly, are struggling to fight the disease on limited budgets.[3] Pooled testing is one potential way to reduce the cost without compromising the accuracy of the tests. The enzyme linked immunosorbent assay (ELISA) protocol for pooled sera is simple. Sera of five individuals are pooled and tested using a single test. If the seroprevalence of HIV is low enough, then there is a high probability that all five individuals in the pool are HIV negative. In this case, a single test can give same information as five tests. If, on the other hand, the test outcome is positive, then individual tests would need to be carried out.[4]

Traditional group testing assumptions consider a binary test outcome. If the test is HIV or syphilis negative, then the pool is released for transfusion. If, however, the test is HIV or syphilis positive, then the pool is tested individually.[3] Various immunological tests are designed to detect antibodies, thereby identifying the serological status of the individual.

An ELISA for HIV detects the HIV p24 antigen or anti-HIV antibodies and is frequently used for HIV screening.[5] Also, an indirect ELISA, to test antibodies against *Treponema pallidum* in human serum/plasma, is used to investigate for syphilis infection.[6]

Thus, the rationale of our study was to investigate the cost-effectiveness and accuracy of pooled sera analysis for both HIV and syphilis in blood donors attending Suez Canal University Hospital Blood Bank compared to their individual sera. We hypothesized that pooled sera testing could reduce the cost and time needed for analysis and yet would give the same accurate results as individual samples analysis.

Materials and Methods

Study population

Samples from blood donor were collected at the central blood bank, Suez Canal University Hospital, Ismailia during January 2021 to October 2021. Laboratory tests were performed at the blood bank serology unit, Clinical Pathology Department of Suez Canal University Hospital, Ismailia, Egypt. Inclusion criteria included both sexes and age between 18 to 65 years. HCV antibodies positive donors and HBsAg positive donors were excluded.

A total of 75 sera were collected from blood donors. These included 10 positive HIV sera, 5 positive syphilis sera and 60 negative sera (for both). They were screened using HIV Ag-Ab and syphilis total antibody by commercial ELISA kits. Screening protocols were done by two sequential steps. At first, samples in pools of five were screened for both HIV and syphilis then, positive pools were individually retested by the ELISA methods again. The positive samples were confirmed by another method, on an automated immunoassay analyzer (Architect i1000SR, Abbott, United States).

For HIV, there were 10 positive samples and 10 pools (each pool consisted of one positive HIV serum and 4 negative sera for HIV). For syphilis, there were 5 positive samples and 5 pools (each pool consisted of one positive syphilis serum and 4 negative sera for syphilis).

The cost of HIV ELISA kit (5 plates, total 480 wells) was 7920 LE. The cost of one pool of 5 samples was calculated as 16.5 LE and the cost of processing 5 samples 82.5 L.E. Similarly, the cost for processing cases for syphilis by ELISA was 16 L.E and 80 L.E, respectively.

Laboratory Investigations

Blood Sampling

A venous blood sample (4 ml) was aseptically collected from each donor into a plain tube for performing virology testing. Finger prick by lancet was done to each donor to estimate Hb level by an automated hemoglobinometer

(Combolab TS made by DiaSpect Medical GmbH, Germany), according to the manufacturer's instructions. Serum samples were separated and stored deep frozen at -20°C until used. They were thawed by warming for a few minutes in a water bath at 40°C (to avoid fibrin precipitation).

Pooling of the samples

Serum samples were pooled and screened as the following protocol: Each pool consisted of five samples (one positive sample and four negative samples). From each sample 50 µl was added and mixed properly. Sera were then added from the mixture to the kit wells. The test procedure was strictly followed according to the manufacturer's instructions. Samples in positive pools were retested individually for both HIV and Syphilis using ELISA Kits. The samples were tested in duplicates by ELISA and further confirmation was done using an automated immunoassay analyzer, as mentioned above.

HIV testing was performed using commercial HIV Ag-Ab ELISA kits (REF 72388, Genscreen™ ULTRA, BIO-RAD Diagnostics, France) and syphilis testing performed using commercial Syphilis Total Ab kits (REF 72531, BIO-RAD Diagnostics, France), according to the manufacturer's instructions. The optical density (OD) of negative and positive controls of both HIV and Syphilis was measured and a cut-off value (COV) calculated. The OD of the specimen was divided by the COV value. Samples with OD less than the COV were considered negative (ratio <1) by Syphilis Total Ab. Samples with OD greater than or equal to the COV (ratio > or = 1) were considered positive by Syphilis Total Ab. The same was applied for HIV.

Statistical analysis

Analysis of data was performed using software MedCalc version 9 (7). Description of quantitative variables was in the form of mean, standard deviation (SD), minimum and maximum. Description of qualitative variables was in the form of numbers (No.) and percent (%). Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that most of data were normally distributed (parametric data) so parametric tests were used for most of the comparisons. Comparison between quantitative variables was carried out by One-way analysis of variance (ANOVA) and pairwise comparisons which was used to test the difference between the means of several subgroups of a variable. Comparison between qualitative variables was carried out by Chi-square test, which was used to test the statistical significance of differences in a classification system (one-way classification) or the relationship between two classification systems (two-way classification). Binary correlation was carried out by Pearson correlation test. Results were expressed in the form of correlation coefficient (r) and *p*-values. A *p* value of <0.05 was considered significant.

Results

There was no difference between mean individual samples ratio (10.78±1.25) and pooled sera ratio of HIV positive cases (10.62±2.08) (*p*>0.05), Table 1.

Table 1. Ratio between individual samples and pooled sera of HIV positive cases by ANOVA test.

Ratio	HIV (n=10)	*P* value
	Mean±SD	
Individual	10.78±1.25	NS
Pool	10.62±2.08	

P > 0.05 is not significant (NS).

There was no difference between mean individual samples OD (2.37±0.27) and pooled sera OD of HIV positive cases (2.34±0.46) (p>0.05), Table 2. Samples with ratio > = 1 were considered positive. Regarding the positive results, there was no difference between positive individual samples (100%) and their positive pool (100%) for HIV (*p* >0.05).

Table 2. OD comparison between individual samples and pooled sera of HIV positive cases by ANOVA test.

OD	HIV (n=10)	*P* value
	Mean±SD	
Individual	2.37±0.27	NS
Pool	2.34±0.46	

P > 0.05 is not significant (NS).

There was significant decrease in the mean cost in one pool of 5 samples (16.50 LE) in comparison to five individual samples of HIV cases (82.50 LE) (p<0.05), with cost reduction 80% using the pooling method technique, Table 3.

Table 3. Comparison between cost of performing individual samples and pooled sera of HIV cases by ANOVA test.

Cost (LE)	HIV (n=10) Mean±(SD	*P* value	Cost reduction %
Individual	82.50±0.00	< 0.0001	80%
Pool	16.50±0.00		

*$P \leq 0.05$ is significant.

Costs of screening protocol at varying rates of seroprevalence were calculated for HIV cases. These assumptions were based on the study findings but not part of the screening protocol. Savings ranged from 5% when seroprevalence was set at 15% to 75% when seroprevalence was set at 1%, Table 4.

Table 4. Cost Savings with pooled sera in HIV cases*.

Prevalence %	Positivity rate per 100 sera	Number of tests done	Total cost	% Reduction in cost with pooling
1	One pool	20 pools	330 L. E	75 %
		5 singles	82.5 L. E	
		Total	412.5 L. E	
2	2 pools	20 pools	330 L. E	70 %
		10 singles	165 L. E	
		Total	495 L. E	
5	5 pools	20 pools	330 L. E	65 %
		15 singles	247.5 L. E	
		Total	577.5 L. E	
10	10 pools	20 pools	330 L. E	30 %
		50 singles	825 L. E	
		Total	1155 L. E	
15	15 pools	20 pools	330 L. E	5 %
		75 singles	1237.5 L. E	
		Total	1567.5 L. E	

*Calculations were based on the assumption that a maximum number of pools will be positive.
20 pools of five sera per pool.

There was no difference between mean individual samples ratio (17 ±5.60) and pooled sera ratio of syphilis positive cases (15.66±4.10) (p>0.05) Table 5. There was no significant difference between mean individual samples OD (2.27±0.74) and pooled sera OD of syphilis positive cases (2.09±0.54) (p>0.05)

Table 5. Ratio between individual samples and pooled sera of syphilis positive cases by ANOVA test.

Ratio	Syphilis (n=5) Mean±SD	*P* value
Individual	17±5.60	NS
Pool	15.66±4.10	

P > 0.05 is not significant (NS).

Table 6. Samples with ratio > = 1 were considered positive. Regarding the positive results, there was no significant difference between positive individual samples (100%) and their positive pool (100%) for syphilis (p>0.05). There was significant decrease of mean cost in one pool of 5 samples (16 LE) in comparison to 5 individual samples within syphilis cases (80 L.E) (p<0.05), with cost reduction 80% with the pooling method Table 7.

Within HIV cases, a comparison of the reactivity ratios (specimen optical density/cutoff optical density) for reactive pooled sera with those for the corresponding individual sera demonstrated a moderate positive correlation between pooled and individual ratios (r= 0.508), suggesting a linear positive relationship but it did not each significance level (p>0.05) Figure 1. The reactivity ratio of HIV was higher for a few pooled sera than their individual sera and most samples showed an unaccountable decrease in reactivity after pooling. Nevertheless, 100% agreement between pooled and individual final HIV antibody test interpretations remained (ratio >= 1) indicate positivity of the sample. Finally, there was significant positive correlation between individual samples ratio and pooled sera ratio (p<0.05) in syphilis cases Figure 1.

Table 6. OD comparison between individual samples and pooled sera of syphilis positive cases by ANOVA test.

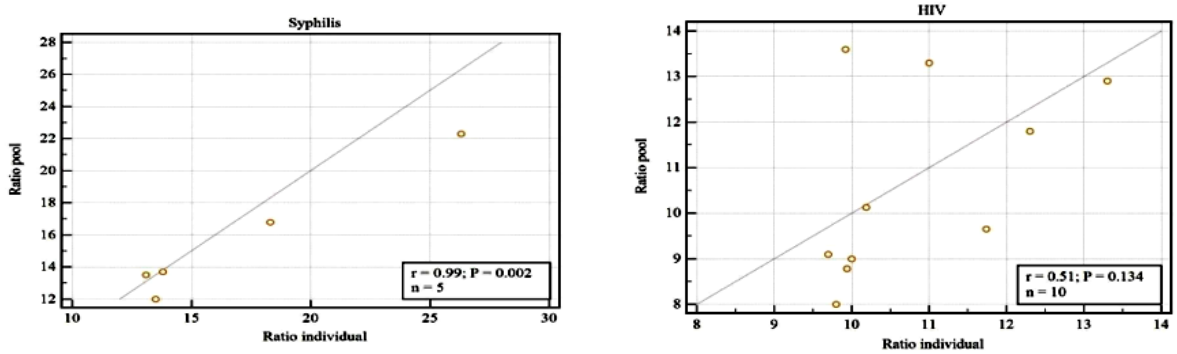
OD	Syphilis (n=5) Mean±SD	*P* value
Individual	2.27±0.74	NS
Pool	2.09±0.54	

P > 0.05 is not significant (NS).

Table 7. Cost Comparison between individual samples and pooled sera of syphilis cases by ANOVA test.

Cost (L.E)	Syphilis (n=5) Mean±SD	**P* value	Cost reduction %
Individual	80±0.00	< 0.0001	80%
Pool	16±0.00		

**P* ≤ 0.05 is significant.



(A) (B)

Figure 1. Pearson Correlation between ratio in pooled sera versus individual samples in HIV and syphilis. (A) Pearson Correlation in HIV cases showed that (R= 0.508, p>0.05) was considered not significant but moderate positive correlated however there were complete agreement in reactivity between individual samples and their pooled sera. (B) Pearson Correlation in syphilis cases showed that (R=0.99, p<0.05) was significant positive correlation.

Discussion

Egypt remains a low HIV prevalence country [2]. The costs of screening blood materials in blood banks are high and are considered financial burden especially in developing countries.

The current study aimed to investigate pooled sera for HIV and syphilis in blood donors compared to their individual sera to reduce the cost taking into account previous studies that demonstrated pooling was feasible and cost-effective even in populations with high prevalence of HBV and HCV infection.[8,9] As far as we know, no previous research has investigated pooling method by ELISA on syphilis. However, in the current study, we

chose only 2 parameters HIV and syphilis infection. There were recommendations by the World Health Organization for HIV where a maximum of five specimens per pool was recommended in areas with seroprevalence rates not higher than 2%.[10] Therefore, pools of five sera would leave a reasonable safety margin in the current study.

In the current study, there was no significant difference in the mean OD between individual samples and their pools for both HIV and syphilis positive cases because of low sized pools used. Complete agreement in reactivity between individual samples and their pooled sera was found because pools were found to be reactive only when the individual specimen was true positive.

In the current study, costs of screening protocol at varying rates of seroprevalence were calculated; pooled serum protocol seems to reduce the costs in developing countries where there is shortage in laboratory chemical supplies.

Emmanuel et al., 1988 found no significant differences between positive pools of five and their individual sera results for HIV using ELISA and cost reduction up to 70 % with pooled serum protocol which agreed with our study. Also, Emmanuel et al., 1988, found no loss of either sensitivity or specificity when they used pools of five samples, however, using pools of 10 samples resulted in loss of sensitivity for low antibody titer specimens.[11]

Monzon et al., 1992 used sera with low, medium, and high antibody titers. The sera were pooled (pools of 5, 10 and 20 samples) with HIV-1 negative sera and tested by ELISA and particle agglutination test. They found that all reactive samples were detected in the pools of five sera in ELISA assay but sera with low antibody titer were not detected in the pools of 10 and 20 samples in ELISA, but they were detected with the particle agglutination method which agreed with our choice of using 5 sample pooling method.[12]

Also, Babu et al., 1993 reported findings using HIV antibody positive sera. The sera were titrated and introduced into pools of 2, 4, 8, 16, 32 or 64 sera in such a manner that each pool had one positive sample and the rest, HIV antibody negative sera. They found that all pools with high titer antibody positive sera were reactive irrespective of pool size by ELISA assay, while some of the pools containing medium or low titer sera were non-reactive when pool size exceeded 16 samples which agreed with our study in using 5 sample pooling sera.[13]

Cahoon-Young et al., 1989 found an approximately 60 to 80% savings in materials when a pooled serum protocol was used for HIV seroprevalence which agreed with the results of our study.[14]

However, a non-significant moderate positive correlation in the current study disagreed with Cahoon-Young et al., 1989 who found a significant good correlation between pooled and individual ratios, suggesting a linear relationship and that could be because of high prevalence of HIV infection in their area and feasibility of positive HIV specimen collection.[14] The findings in the current study are consistent with those obtained by other investigators who applied the method of pooling to the study of HBsAg, HCV and HTLV infections.

Fernandez et al., 2009, found that the technique of pooling sera for the detection of HBsAg was highly sensitive and specific (100%), and the cost- benefit analysis showed that the pooling method could save from 30% up to 75% of the cost of HBsAg screening, according to whether seroprevalences were 10% or 1%, respectively. So, the pooled HBsAg EIA was a cost-effective and valid strategy in areas with a high, medium, or low prevalence. Also, they found that the application of the pooling method could save around 74% of total economic costs when compared to the single method in their area which agreed with our study.[15]

Fernandez et al., 2009 found significant correlation among the ratios of the samples studied in single and in pooled conditions. Also, they found that the cutoff on the test kits could be lowered so that borderline results are called positive and confirmed by retesting individually to decrease the false negative results in pooled sera.[15]

Cunningham et al., 1998 aimed to validate antenatal screening for HBsAg by pooled sera protocol. The sera were tested individually and

in pools of 10 sera for HBsAg by ELISA assay to identify women who were likely to transmit HBV infection to their infants. In their study, HBsAg ELISA assay was highly sensitive, so, HBV carriers had concentrations of HBsAg to be detected and a false negative result would be an extremely rare event. Also, they found the chance of vertical transmission resulting from a false negative screening for HBsAg would be substantially less than 10% because of low prevalence of hepatitis B infection in the antenatal population in their area.[16]

Novack et al., 2008 found the sensitivity of pooled testing for HBsAg was 93- 99%, and serological testing for HBsAg could be performed using manually created pools up to six samples, with 5% loss in sensitivity in the donor population at Israeli national blood bank and Shifa hospital blood bank in Palestinian Authority. So, pooling could be considered as an option only in countries with a low prevalence of HBV infection.[17]

Novack et al., 2007 performed experiments with manually created pools of 24, 12 and 6 samples for anti-HCV antibodies. They found that the sensitivity of pooled sera testing for anti-HCV was 96- 97%, cost-analysis showed benefits up to $2 per donation with screening of anti-HCV in pools of 6 samples and the specificity of pooled sera testing was 93-100%. Also, they found that screening in large pools needed fewer tests per donation, but provided lower sensitivity, and therefore, a higher false negative rate. They recommended using manually created pools of up to 6 samples in the screening of anti-HCV, with 3% loss in sensitivity which agreed with our study.[9]

da Silva et al., 2020 found diagnostic sensitivity (100%) and specificity (100%) of the pooling method when pools of five samples were used. Also, there was a cost minimization varying from 60.7% to 73.6% and that was because of the high prevalence of HTLV infection in their area [18] Andersson et al., 2001 evaluated pooling strategy for antibody screening of HTLV-I/II by ELISA assay. Each HTLV-positive sample was included in pools of 1/1, 1/2, 1/4, 1/8 and 1/16 mixed with HTLV-negative sera. They found gradually decreasing sensitivity for HTLV from 98 % (1/1) to 33% (1/16). They recommended using pools of five samples in screening of HTLV.[19]

In conclusion, screening protocols using pooling method seems to reduce the cost in developing countries where there is shortage in laboratory chemical supplies and low prevalence of HIV and syphilis infection. Pooling serum testing appears to be a reliable and economical method for screening HIV and syphilis infection.

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Author Contributions

FMA and MHSM conceived and designed the experiment. MAG recruited the study samples. MAG and MHSM contributed to the practical experiment. HAK contributed to the statistical analysis. MAG contributed the reagents and materials needed for the current work. MAG, MHSM, HAK, FMA contributed in writing and approval of final manuscript.

Declaration of Conflicting Interests

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Ethical approval

The protocol of the study was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Suez Canal University (no 4377 dated November 2020).

Informed consent

A signed consent form was obtained from each study participant.

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