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Plasma pool testing for viral markers. Validation report

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Plasma pool testing for viral markers. Validation report

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The validity of employing in plasma pool testing commercial kits for virus serological control was assessed spiking three plasma pools with virus positive material. Test linearity, sensitivity and accuracy for HBsAg, antibody anti-HIV 1/2 and anti-HCV were determined. The overall data demonstrate that commercial kits designed and authorised for single serum or plasma may be also employed for plasma pool testing.
Key words: HBsAg, HCVAb, HIV 1/2 Ab, Plasma pool, Virus markers

Istituto Superiore di Sanità
Ricerca dei marcatori virali nei pool di plasma. Rapporto di validazione.
Claudio Mele, Maria Puccinelli, Guillermo Bisso e Giuliano Gentili
2000, 13 p. Rapporti ISTISAN 00/15 (in inglese)

L'impiego di kit commerciali per la ricerca di marcatori virali nel controllo dei pool di plasma è stato convalidato contaminando tre pool di plasma con materiale virale positivo. E' stata valutata la linearità, sensibilità e accuratezza dei saggi impiegati. I dati complessivi dimostrano che i kit commerciali sviluppati ed autorizzati per singole donazioni possono essere utilizzati anche per il controllo dei pool di plasma.
Parole chiave: HBsAg, HCVAb, HIV 1/2 Ab, Marcatori virali, Pool di plasma

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INTRODUCTION

The possibility of transmitting viral infection by blood products continues to be a matter of concern for health authorities. Plasma pool testing for virus markers represents one of the steps in the strategy implemented to guarantee the safety of blood products. The European Pharmacopoeia has established that during the manufacture of blood products, the first homogeneous plasma pool must be tested and found negative for: the surface antigen of hepatitis B virus (HBsAg), the antibody anti-hepatitis C virus (antibody anti-HCV) and the antibody anti-human immunodeficiency virus (antibody anti-HIV1/2), using methods of suitable sensitivity and specificity (1).

In compliance with such requirements, the Section Prodotti Immunologici has introduced plasma pool testing in the procedure for Batch Release of immunoglobulins (2). Since the adopted methods employ commercial kits designed and authorised only for individual serum or plasma samples, it appeared necessary to validate their use in plasma pool testing (3).

1. SCOPE

The study was aimed to assess the possibility and validity of employing commercial kits for the virus serological control of plasma pools taking into account that they constitute a very variable matrix and therefore, false results may occur. Moreover, as they are prepared by pooling a large number of single donations, up to 30,000, it is almost certain that only those donations with a high titre for virus markers would be surely detected, due to dilution effects.

The linearity, the sensitivity and the accuracy of the tests for the three virus markers HBsAg, antibody anti-HIV1/2 and antibody anti-HCV were determined. Three different plasma pools, originated from different countries were spiked with virus positive material of two different levels of reactivity.

2. MATERIALS

Plasma pools

- Sample 1 (USA)
- Sample 2 (Europe)
- Sample 3 (Europe)

All tested negative for HBsAg, antibody HIV1/2, antibody anti-HCV and positive for antibody anti-HBsAg.

- Single donation for reference control.

Tested negative for HBsAg, antibody HIV1/2, antibody and anti-HCV antibody anti-HBsAg.

HBsAg

Spike material

a) strong reactive (presumptive titre 1:250,000)
b) weak reactive (presumptive titre 1:10,000)

Antibody anti-HIV

Spike material

a) reactive I (presumptive titre 1:14,000)
b) reactive II (presumptive titre 1:10,000)

Antibody anti-HCV

Spike material

a) strong reactive (presumptive titre 1:800)
b) weak reactive (presumptive titre 1:200)

Commercial kits:

- AUSZYME^{R} MONOCLONALE ABBOTT, a qualitative third generation enzyme immunoassay for the detection of Hepatitis B Surface Antigen (HBsAg) in human serum or plasma.

- ABBOTT HIV-1/HIV-2 3rd GENERATION PLUS EIA, an *in vitro* enzyme immunoassay for the simultaneous detection of antibodies to Human Immunodeficiency Viruses type 1 and/or type 2(HIV-1/HIV-2) in human serum or plasma.

- ABBOTT HCV EIA 3.0, an enzyme immunoassay for the qualitative detection of antibody to Hepatitis C Virus (anti-HCV)in human serum or plasma.

3. METHODS

Test sample preparation

Test solutions for each virus marker were prepared by serially diluting each spike material separately with the three plasma pool samples and the negative single donation. The latter becomes, thus, a positive reference control test solution. The resulting dilutions were the following:

HBsAg:

a) spiked with strong reactive material
   1:32,000; 1:64,000; 1:128,000; 1:256,000; 1:512,000
b) spiked with weak reactive material
   1:2,000; 1:4,000; 1:8,000; 1:16,000; 1:32,000

Antibody anti-HIV:

a) spiked with reactive material I
   1:2,048; 1:4,096; 1:8,192; 1:16,384: 1:32,768
b) spiked with reactive material II
   1:2,048; 1:4,096; 1:8,192; 1:16,384: 1:32,768

Antibody anti-HCV:

a) spiked with strong reactive material:
   1:128; 1:256; 1:512; 1:1,024; 1:2,048
b) spiked with weak reactive material:
   1:32; 1:64; 1:128; 1:256; 1:512

For each virus marker, and for each level of spike material four series of the above test solutions, one for each plasma pool and one for the negative single donation, were tested.

Assay procedure

For each virus marker, test solutions were assayed following the producer's specifications of the respective commercial kits. Considering that the analyte may be alternatively an antigen (HBsAg in AUSZYME^{R} MONOCLONALE ABBOTT) or an antibody (anti-HIV in ABBOTT HIV-1/HIV-2 EIA PLUS or anti-HCV in ABBOTT HCV EIA 3.0), the three immunoenzymatic methods are based on a common principle which may be briefly summarised as follows:

Polystyrene beads coated with the respective counter-analyte are incubated with the test sample and suitable controls. The analyte, if present, will bind to the respective counter-analyte forming a complex antigen-antibody. After removing the unreacted

plasma by washing, the beads bearing the complex antigen-antibody are allowed to react either with 1) an antibody anti-analyte in AUSZYME^{R} MONOCLONALE ABBOT, or 2) recombinant HIV proteins in ABBOTT HIV-1/HIV-2 EIA PLUS or 3) anti-human immunoglobulin in ABBOTT HCV EIA, all of them conjugated to horseradish peroxidase. Unbound enzyme conjugated is then aspirated and the beads are washed. Next, o-phenylendiamine solution containing hydrogen peroxide is added to the beads and after incubation, a yellow-orange develops in proportion to the amount of the bound analyte.

Statistics

A descriptive statistics of plasma pool size of samples tested at the Section Prodotti Immunologici in the period 1996-1999, is reported.

Linearity of test reaction was assessed by regression of the logarithm of Fn versus the logarithm of reciprocal dilution of the experimental data.

The Fn is defined by the formula:

$$Fn = \frac{O.D.}{C.O.}$$ where:

O.D. = sample optical density;

C.O. = cut-off value.

Test accuracy was evaluated determining the percentage of recovery at the detection limit:

$$\% \text{ recovery} = \frac{Fdl}{\overline{F}dl} \times C.F. \times 100$$, where:

Fdl = the lowest value of Fn above 1;

$\overline{F}dl$= mean of Fdl of the 3 pool samples and single donation;

$$C.F \text{ (correction factor)} = \frac{\text{Fdl reciprocal dilution pool sample}}{\text{Fdl reciprocal dilution single donation}}$$

The value of Fn = 1 corresponds to what is called "detection limit" above which a reaction is considered positive.

Such a formula appears valid considering that the starting concentration of spike material was the same for the 4 samples, and that the detection limit should indicate an identical minimal concentration.

To evaluate test sensitivity, Fn of those reciprocal dilutions corresponding to the most frequent pool sizes recorded during 1996-1999 were interpolated from the linear regression equations, assuming that the latter are still valid beyond the range of tested dilutions.

4. RESULTS AND DISCUSSION

Plasma pool composition

Figure 1 shows the pool size frequency of plasma pools examined in the period 1996-1999. From a total of 1,922 samples pool size ranged from 860 to 26,000 donations (mean ± SE:5,527 ± 97).

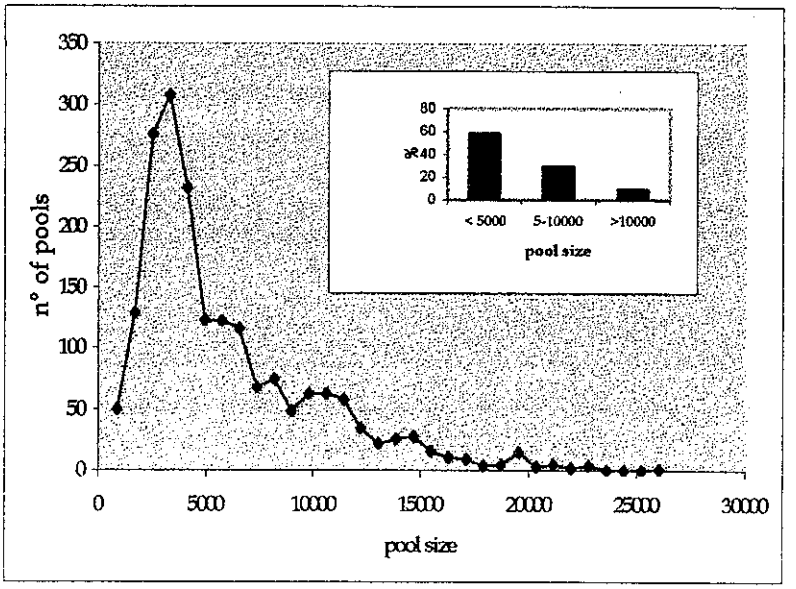

Figure 1.- *Pool size frequency during 1996-1999*

Three different groups can be distinguished: pools of not more than 5,000 single donations (59%), those constituted by a n° of donations comprised between 5,000 and 10,000 (26%) and pools containing more than 10,000 donations (15%). This means that a virus positive donation potentially present in one of the plasma pools analysed would have had a probability of 59% to be diluted up to 1:5,000 of 26% up to 1:10,000 and of 12% beyond this dilution. Therefore, the figures 5,000 and 10,000 indicating the two upper limit size of over 85% of plasma pools analysed during the whole period of the study, were taken as those reciprocal dilutions at which Fn should be interpolated to evaluate test sensitivity.

HBsAg

Table 1 shows Fn values and the respective dilutions corresponding to the three plasma pool samples and to the positive reference control obtained with AUSZYME^{R}

MONOCLONALE ABBOT from both levels of spiked test solutions. The correlation coefficients of the linear regression of log Fn versus log reciprocal dilution were comprised between 0.978 and 0.998 demonstrating that linearity of the test is guaranteed all over the measuring range.

A very strong positive donation, presumptive titre 1:250,000, would be easily detected whether in a pool of 5,000 (Fn=25) or of 10,000 (Fn=13) donations. Conversely, a weakly positive donation, presumptive titre 1:10,000, would be masked by dilution effects in a pool of 10,000 donations (Fn=0.6), but detected in a pool of 5,000 donations (Fn=1.1).

A poor recovery was observed in both series of spiked test solutions, 54.3% and 52.3% for weak and strong positive material, respectively indicating some loss of test sensitivity. This can be explained by the presence in plasma pool samples of antibodies anti-HBsAg which partially neutralising the added HBsAg would decrease its ability to be detected. In the present study the mean antibody content of three plasma pool was: 103 ± 28 mU/ml.

Table 1.-*a) Test solutions spiked with strong reactive material*

Dilution	Fn			
	Single plasma	Plasma pool n°1	Plasma pool n°2	Plasma pool n°3
1/32,000	7.41	4.61	4.96	5.22
1/64,000	3.35	2.44	2.30	2.80
1/128,000	1.81	1.18	1.28	1.26
1/256,000	1.05	0.67	0.55	0.74
1/512,000	0.76	0.37	0.31	0.35
r (n=5)	0.978	0.998	0.997	0.997
1/5,000	30	25	31	32
1/10,000	17	13	16	16
% Recovery		50%	54%	53%
		Mean ± SD: 52.3 ± 2.1 CV: 4%		

Table 1.-*b) Test solutions spiked with weak reactive material*

Dilution	Fn			
	Single plasma	Plasma pool n°1	Plasma pool n°2	Plasma pool n°3
1|2,000	4.30	2.65	2.89	2.85
1/4,000	2.15	1.37	1.65	1.46
1/8,000	1.02	0.70	0.70	0.59
1/16,000	0.55	0.43	0.44	0.39
1/32,000	0.33	0.18	0.18	0.17
r (n=5)	0.995	0.994	0.992	0.991
1/5,000	1.7	1.1	1.2	1.1
1/10,000	0.9	0.6	0.6	0.6
% Recovery		50%	60%	53%
		Mean ± SD: 54.3 ± 5.1 CV: 9.5%		

Figure 2 shows the plots of log Fn versus log reciprocal dilution corresponding to the three plasma pool samples, spiked with strong and weak positive material, which appear to be satisfactorily parallel to that of the positive reference control. They are, however, significantly shifted down, confirming the above mentioned loss of test sensitivity.

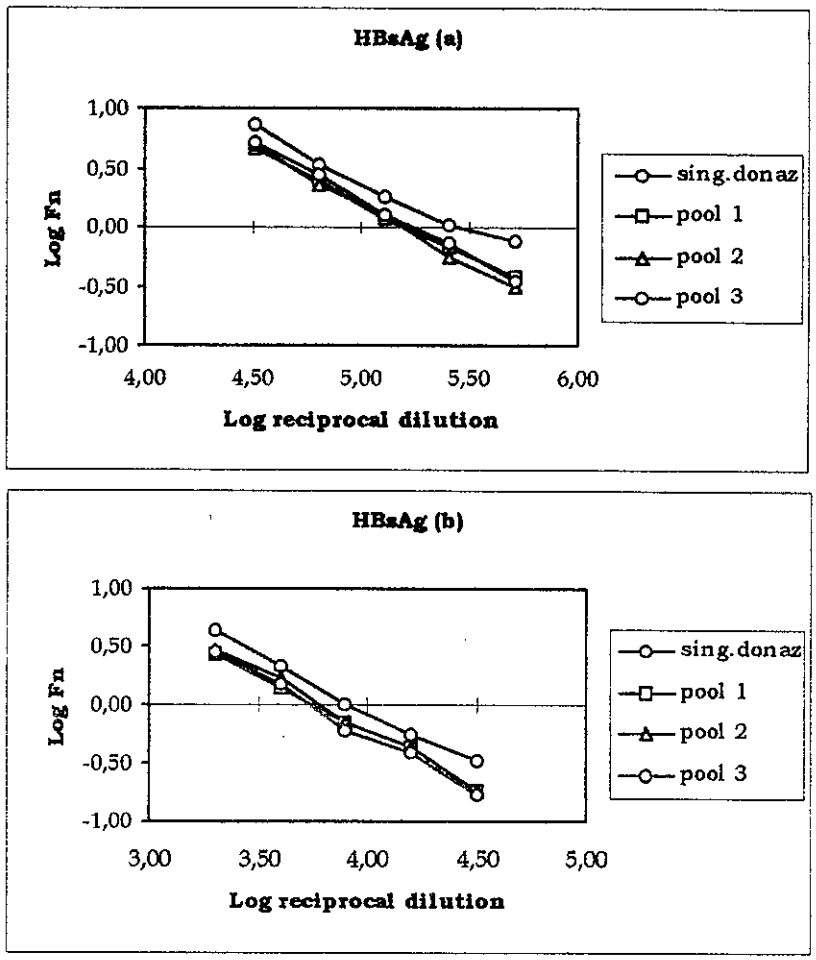

Figure 2 - HBsAg. Plots of log Fn versus log reciprocal dilution from the experimental data spiked both types of HBsAg material (**a**: strong spike; **b**: weak spike).

Antibody anti-HIV

Table 3 shows Fn values and the respective dilutions corresponding to the three plasma pool samples and to the positive reference control obtained with ABBOTT HIV-1/HIV-2 EIA PLUS from both levels of spiked test solutions. The correlation coefficients of the linear regression of log Fn versus log of reciprocal dilutions were comprised between 0.996 and 0.999 showing a very good linearity of the test all over the measuring range.

Table 2.-*a) Test solutions spiked with reactive material I*

Dilution	Fn			
	Single plasma	Plasma pool n°1	Plasma pool n°2	Plasma pool n°3
1/2,048	4.67	4.74	5.27	5.08
1/4,096	2.72	2.92	2.95	2.94
1/8,192	1.51	1.60	1.70	1.70
1/16,384	0.90	0.92	1.03	1.07
1/32,768	0.56	0.60	0.63	0.66
r (n=5)	0.999	0.998	0.999	0.999
1/5,000	2.31	2.41	2.58	2.56
1/10,000	1.35	1.42	1.52	1.54
% Recovery		94%	105%	109%
		Mean ± SD: 103 ± 7.8 CV: 7.5%		

Table 2.-b*) Test solutions spiked with reactive material II*

Dilution	Fn			
	Single plasma	Plasma pool n°1	Plasma pool n°2	Plasma pool n°3
1/2,048	4.68	4.98	4.83	4.75
1/4,096	2.43	2.66	2.67	2.74
1/8,192	1.44	1.43	1.47	1.58
1/16,384	0.89	0.90	0.92	0.91
1/32,768	0.51	0.57	0.59	0.62
r (n=5)	0.998	0.996	0.997	0.998
1/5,000	1.98	2.10	2.10	2.15
1/10,000	1.15	1.20	1.20	1.30
% Recovery		97%	99%	106%
		Mean ± SD: 101 ± 4.7 CV: 4.75%		

No masking dilution effects were observed. In fact, positive donations with titres 1:10-14,000 would be surely detected both in a pool of 5,000 (Fn= 2.10-2.58) or 10,000 (Fn= 1.20-1.54) donations. A good recovery for both groups of spiked test solutions was found: 103%; and 101%.

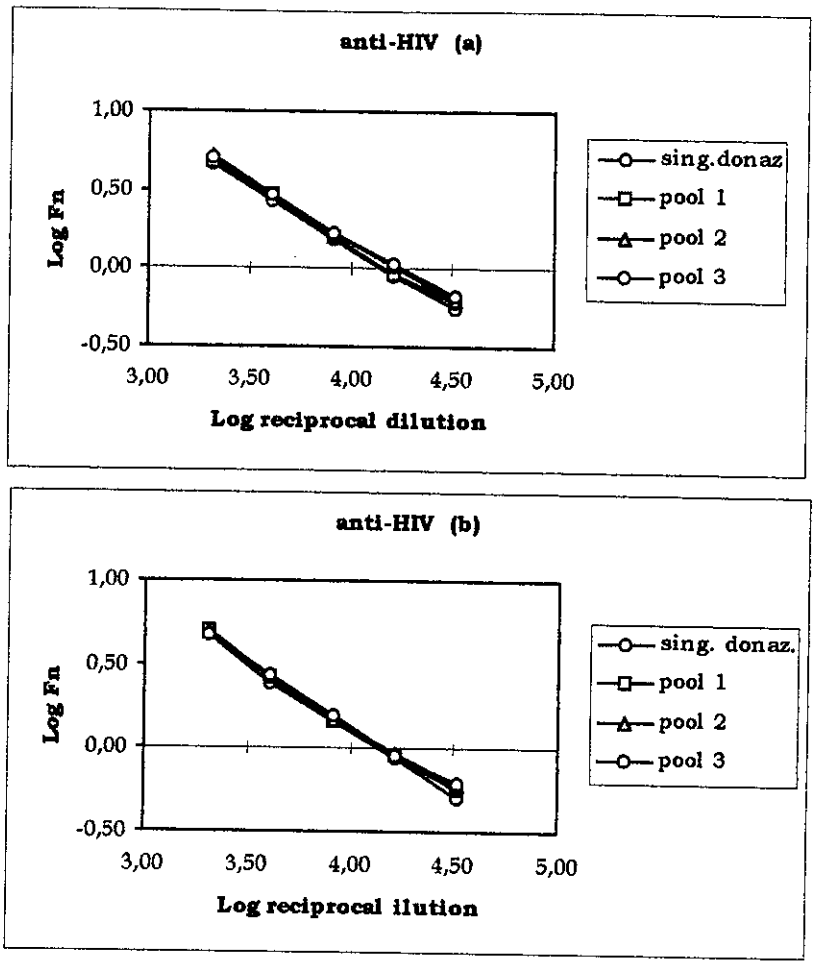

Figure 3 – Antibody anti-HIV. Plots of log Fn versus log reciprocal dilution value from the experimental data of pools spiked with both types of HIV material (**a**: spike material I; **b**: spike material II).

As shown in figure 3, the plot lines of log Fn versus log reciprocal dilutions corresponding to the three plasma pool samples overlapped each other with that of the positive reference control.

No shifting at all was observed. This finding and the overall satisfactory parallelism of plot lines are consistent with the good recovery mentioned above confirming that no loss of test sensitivity occurred.

Antibody anti-HCV

Table 3 shows Fn values and the respective dilutions corresponding to the three plasma pool samples and to the positive reference control obtained with ABBOTT HCV

EIA from both levels of spiked test solutions. For this test, linearity was guaranteed all over the measuring range as demonstrated by the correlation coefficients of the linear regression of log Fn versus log of dilution, which were comprised between 0.985 and 0.999.

A detection limit ranging between a maximum reciprocal dilution of 500-1,000 for strong positive spiked material and less than 250 for a weak one, was found. Therefore, either a strong (titre 1:800) or a weak (titre 1:200) positive donation would have escaped detection in a pool of 10,000 (Fn=0.16-0.04) as well as in one of 5,000 (Fn =0.21-0.07) donations.

Table 3.-*a) Test solutions spiked with strong reactive material*

Dilution	Fn			
	Single plasma	Plasma pool n°1	Plasma pool n°2	Plasma pool n°3
1/128	4.06	4.87	4.24	5.05
1/256	2.43	2.21	2.18	2.49
1/512	1.37	1.38	1.42	1.41
1/1,024	0.75	0.71	0.79	0.81
1/2048	0.43	0.50	0.54	0.54
r (n=5)	0.999	0.985	0.992	0.991
1/5,000	0.21	0.21	0.10	0.10
1/10,000	0.12	0.12	0.16	0.14
% Recovery		99%	102%	101%
		Mean ± SD: 101.5 ± 1.5 CV: 1.5%		

Table 3.-*b) Test solutions spiked with poor reactive material*

Dilution	Fn			
	Single plasma	Plasma pool n°1	Plasma pool N°2	Plasma pool n°3
1/32	5.80	5.19	5.47	5.89
1/64	2.99	3.22	3.35	3.10
1/128	1.53	1.57	1.57	1.95
1/256	0.81	0.76	0.94	1.02
1/512	0.60	0.56	0.56	0.63
r (n=5)	0.984	0.985	0.994	0.997
1/5,000	0.08	0.07	0.08	0.10
1/10,000	0.04	0.04	0.04	0.06
% Recovery		95%	95%	117%
		Mean ± SD: 102 ± 12.7 CV: 12.5%		

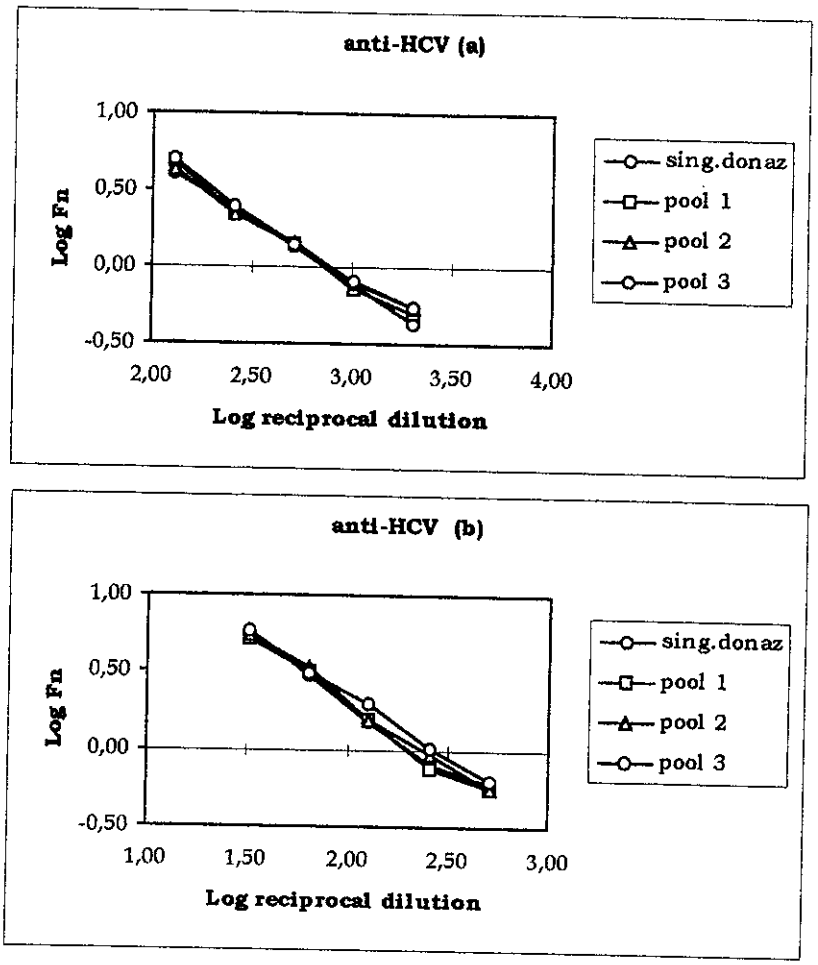

Figure 4 – Antibody anti-HCV. Plots of log Fn versus log reciprocal dilution value from the experimental data of pools spiked with both types of HCV material (**a**: strong spike material; **b**: weak spike material).

For the three plasma pool test solutions a good recovery was found 102%, and 101% for weak and strong spiked samples, respectively. A similar finding is also deduced from figure 4, where the respective plots of log Fn versus log reciprocal dilution showed practically overlapping lines with that of the positive reference control .

5. CONCLUSIONS

The overall data presented here indicate that the testing of human plasma pools for virus markers using commercial kits, designed and authorised for single serum or plasma samples, substantially shows no conditional restrictions, except the obvious dilution of the analytes implied in manufacturer's pooling strategy. This is particular true as regards ABBOTT HIV-1/HIV-2 EIA PLUS and ABBOTT HCV EIA 3 while, in the case of AUSZYME^{R} MONOCLONALE ABBOT the significant decrease of titre found for all samples means that the presence in plasma pools of variable amounts of antibodies antibody anti-HBsAg may affect test sensitivity.

It is worthwhile to remember that plasma pool testing is not aimed to the identification of a virus contaminated donation potentially present in the pool. It serves instead, to assess compliance with European Pharmacopoeia requirements of plasma pool quality. Keeping this in mind, the present report shows anyway that: for HBsAg, a donation with low titre (1:10,000) would have been detected in 59% of the 1996-1999 pool samples, while detection level for those with high titre (1:250,000) would have risen to 100%. The risk of a variable decrease of test sensitivity due to different levels of antibody anti-HBsAg in plasma pools, however, does not allow to generalise this forecast. In the case of antibody anti-HIV1/2, it would have been possible to detect positive donations with titre above 1:8,000 in 85% of the above mentioned samples. On the other hand, for antibody anti-HVC the detection limit found with the spike material employed being much lower than the mean pool size, one can just hypothesise that to reach an adequate the detection degree of positive donations should have titres exceeding such pool size.

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