The Influence of Blood Taking, Storage Conditions and Interfering Factors on a New Fibrin Monomer Test

Der Einfuß von Blutentnahme, Probenlagerung und Störfaktoren auf einen neuen Fibrinmonomer-Test

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Summary: We studied the influence of venous occlusion, sample storage and possible interfering factors on a new fibrin monomer test, Enzymun-Test® FM. The venous occlusion study was performed in healthy volunteers (8 males and 2 females, aged 25-40 years). A manometer cuff on the upper arm was inflated above diastolic pressure and a cubital vein punctured. Blood (2 ml void followed by 4.0 ml mixed with citrate solution at 10:1 ratio) was taken immediately after puncture and at 1, 3 and 5 minutes after inflation of the cuff. Then the pressure was released and further blood samples were taken at 5.5 and 8 min. Samples were also taken with the same occlusion scheme, but with a single puncture taken at 5 min or with no occlusion at all, but with the needle in place. Aliquots of the samples were stored 0.5, 1.2, 3, 6.5 and 23 hours at room temperature as whole blood. The storage of citrate plasma was studied at room temperature, 4 and -20 °C. The effects of added bilirubin, hemolysate and triglyceride were investigated with samples of healthy subjects and patients. Our results showed no relevant differences between immediate venous blood draw and different times of occlusion. Blood drawing after more than 5 minutes after setting the occlusion and also after release of the pressure should be avoided. Different storage conditions and possibly interfering factors with exception of hemolysis had no relevant effect.

Key words: Fibrin Monomer/plasma; Blood Specimen Collection; Specimen Handling; False Positive Reactions.

Zusammenfassung: Wir untersuchten den Einfluß von venöser Stauung, Lagerungsbedingungen und potentiellen Störfaktoren auf einen neuen Fibrinmonomer-Test, Enzymun-Test® FM. Bei gesunden Probanden (8 Männern) und 2 Frauen im Alter von 25-40 Jahren) wurde zur Untersuchung der Abnahmebedingungen ein Stauversuch durchgeführt. Eine Blutdruckmanschette wurde über den diastolischen Druck aufgepumpt und die Vena cubitalis punktiert. Blut (2 ml Totvolumen, gefolgt von 4 ml Blut, das mit Citratlösung im Verhältnis 10:1 gemischt wurde) wurde sofort und 1, 3 und 5 Minuten nach Okklusion entnommen. Nach dem Öffnen wurde 5,5 und 8 Minuten nach Stauungsbeginn weiteres Blut entnommen. Um den Einfluß der liegenden Nadel im Blutgefäß zu klären, wurden zudem unter denselben Stauungsbedingungen Blutproben nach 5 Minuten durch Einfachpunktion oder ohne Stauung aber bei liegender Nadel entnommen. Aliquote Teile der Proben wurden 0,5, 1,2, 3, 6,5 und 23 Stunden bei Raumtemperatur als Vollblut gelagert. Daneben wurden die Lagerungsbedingungen von Citratplasma bei Raumtemperatur, 4 und - 20 °C untersucht. Der Einfluß von zugesetztem Bilirubin, Hämolysat und Triglyceriden wurde bei Proben von gesunden Personen und Patienten geprüft. Unsere Daten zeigen, daß bei sofortiger venöser Blutabnahme und nach unterschiedlichen Okklusionzeiten keine relevanten Unterschiede gefunden werden. Blutentnahmen nach mehr als 5 Minuten Stauung oder nach Freigabe der venösen Okklusion sollten vermieden werden. Unterschiedliche Lagerungsbedingungen und die geprüften Störfaktoren mit Ausnahme von Hämolyse hatten keinen relevanten Einfluß.

Schlüsselwörter: Fibrinmonomere/Plasma; Blutentnahme; Probenbehandlung; Falsch positive Ergebnisse.

Hemostaseologic parameters are easily influenced by the conditions under which blood samples are obtained, stored and analyzed [1]. We therefore studied the influence of blood sampling procedure, sample storage and interfering factors on a new fibrin monomer test.

Methods and Materials

FM concentrations were measured by means of the Enzymun-Test® FM (Boehringer Mannheim GmbH, Mannheim, Germany), evaluation lot E04. The method is based on a ELISA 2-step-sandwich-assay using

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streptavidin-technology. The same fibrin specific monoclonal antibody 2B5 is used both as biotinylated capture antibody and as peroxidase-labelled antibody. The assay is developed for the automated Enzymun-Test® systems ES 300 and ES 33 (Boehringer Mannheim GmbH). Manual performance is also possible [2]. The technical performance data (precision, low detection limit, dilution linearity) were established on ES 300 and ES 33. In addition, a comparison of ES 300 to the ES 700 analyser was carried out on plasma samples obtained from 95 patients.

The effects of blood taking conditions were investigated with healthy volunteers. A manometer cuff on the upper arm was inflated above diastolic pressure and a cubital vein punctured. Blood (2 ml void followed by 4.0 ml mixed with citrate solution at 10:1 ratio) was taken immediately after puncture and at 1, 3 and 5 minutes after inflation of the cuff. Then the pressure was released and blood taken immediately (5.5 min) and again at 8 min after setting the occlusion. Samples were also taken with the same occlusion scheme [3], but with a single puncture taken at 5 min or with no occlusion at all, but with the needle in place, to see the effect of the needle in the vessel.

Aliquots of the samples were stored 0.5, 1.2, 3, 6.5 and 23 hours at room temperature as whole blood. The storage of citrate plasma was also studied at room temperature, 4 °C and -20 °C. The effects of bilirubin, hemolysis and triglyceride as possibly interfering factors were investigated by addition experiments using samples from healthy subjects and patients with activated coagulation system.

Blood samples were obtained from healthy volunteers (8 males and 2 females, aged 25-40 years) and 95 patients after surgical treatment.

Samples from the blood taking study as well as from the storage experiments were analysed on ES 700 which showed comparable results with respect to the ES 300 system.

Results and discussion

Technical Performance Results

Precision:

In a precision study ES 300 and ES ES 33 showed the performances given in table 1 and 2.

Low detection limit (Standard A, mean plus 3 standard deviations):

ES 300 0.17 µg/ml

Es 33 0.42 µg/ml

Dilution linearity:

Up to 62.2 µg/ml for both ES 300 and ES 33.

Comparison study:

The highest FM concentration within the group of 95 patients determined by ES 300 was 240.6 µg/ml, the median was 10.1 µg/ml, the 90th percentile was 55.5 µg/ml and the 10th percentile 0.6. In ES 700 measurement the highest value was 251.9 µg/ml, the median

Table 1 Within-run-precision of FM measurement on ES 300 and ES 33

FM concentration level	Coefficient of variation (%)	
	ES 300	ES 33
Low (4.5 µg/ml)	7.1	13.2
Medium (14.9 µg/ml)	2.2	7.5
High (62.2 µg/ml)	2.1	4.4

Table 2 Run-to-run-precision of FM measurement on ES 300 and ES 33

FM concentration level	Coefficient of variation (%)	
	ES 300	ES 33
Low (3.9 µg/ml)	8.9	12.1
Medium (16.2 µg/ml)	5.0	5.7
High (62.2 µg/ml)	3.1	5.2

Table 3 Influence of various bilirubin additions on measured fibrin monomer concentrations in citrated plasma

Patient/ volunteer	Sample	FM conc. (µg/ml)	Bilirubin conc. (mg/dl)
Volunteer B	Native	0.4	0.3
	+ bilirubin low	0.3	1.5
	+ bilirubin middle	0.4	9.2
	+ bilirubin high	0.3	20.5
Volunteer C	Native	1.9	0.3
	+ bilirubin low	2.1	4.9
	+ bilirubin middle	2.1	12.6
	+ bilirubin high	2.4	19.6
Patient 1	Native	34.9	0.1
	+ bilirubin low	36.8	6.8
	+ bilirubin middle	36.9	17.9
Patient 2	Native	37.8	0.1
	+ bilirubin low	37.7	4.0
	+ bilirubin middle	37.9	20.6

Table 4 Influence of various triglyceride concentrations on measured fibrin monomer concentration in citrated plasma

Patient/ volunteer	Sample	FM conc. (µg/ml)	Tirgleride conc. (mg/dl)
Volunteer C	nativ	0.8	64
	1+1#	1.1 (1.6)*	339
	1+2§	1.8 (1.9)*	422
Patient 1	native	44.2	371
	1+1#	23.7 (22.3)*	448
	1+2§	15.8 (16.3)*	
Patient 2	native	32.0	50
	1+1#	16.9 (17.2)*	296
	1+2§	12.3 (12.3)*	363

#,§ Addition of 1 resp. 2 parts of lipemic plasma (FM conc. 2.4 µg/ml).
* Nominal value.

Nonstandardard abbreviations: CV, coefficient of variation; FM, Fibrin monomer.

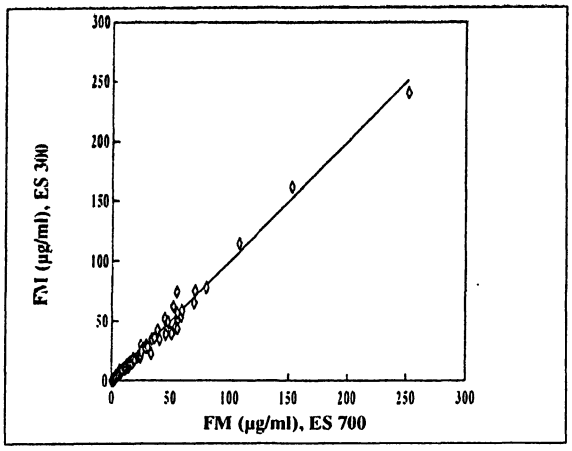

Fig. 1 Comparison of FM values obtained with ES 300 or ES 700 (r = 0.993, slope = 0.997, intercept = -0.876, n = 95).

was 11.8 µg/ml, the 90th percentile was 55.7 µg/ml and the 10th percentile 1.1. The regression analysis revealed a very good correlation of ES 300 versus ES 700 results (r = 0.993, slope = 0.997, intercept = -0.876, n = 95) (Fig. 1).

Blood taking conditions

FM values are largely unaffected by our procedure, an increase can only be observed when the occlusion is opened (Fig. 2a,b).

Samples were additionally taken with the same occlusion scheme, but with a single puncture taken at minute 5 (Fig. 4) or with no occlusion at all (Fig. 3) but with the needle in place to see the effect of the needle in the vessel. No change in fibrin monomer concentration was found during the time of occlusion. Also the needle in place had no effect on the monomer values.

Storage conditions

Aliquots of the samples were stored 0.5, 1.2, 3, 6.5 and 23 hours at room temperature as whole blood (Fig. 5a-c). Additionally, the storage of citrate plasma was studied at room temperature, 4 °C and -20 °C (Fig. 6a,b). We found no relevant influence on fibrin monomer concentrations.

Interfering Factors

As possible interfering factors bilirubin, hemolysis, triglyceride were investigated in healthy subjects and patients. Elevated bilirubin (Table 3), triglyceride (Table 4) do not act as interfering factors. Hemolysis leads to a slight increase of the values, therefore samples with visible hemolysis have to be discarded (Table 5).

Enzymun-Test® FM shows good technical performance on ES 300 and ES 33. The use of ES 700 is not recommended by the manufacturer since actual measurement of FM values would result small analytical series where the large instrument is not appropriate. However, the good correlation of ES 700 with ES 300,

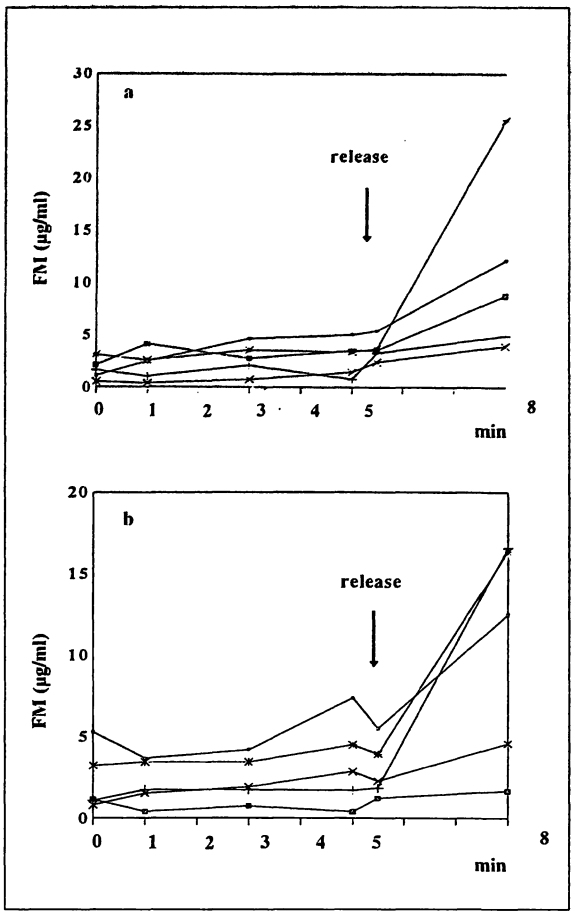

Fig. 2 a, b Time course of fibrin monomer concentration in citrated plasma samples of 10 healthy volunteers with laying needle during venous occlusion (5 min, 80 mm Hg) and after release of pressure (arrow). -■-, -+-, -✳-, -□-, -×-, healthy proband number 1-5 (Fig 2a) or 6-10 (Fig 2b).

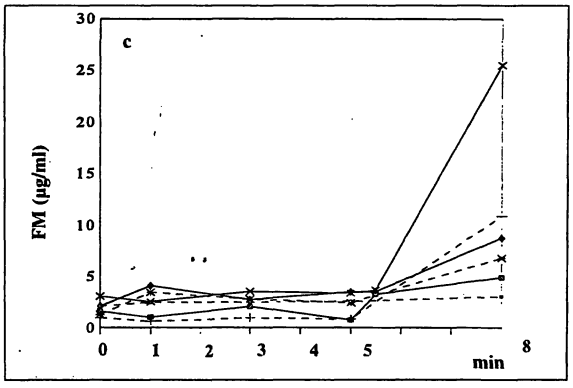

Fig. 3 Time course of fibrin monomer concentration in citrated plasma of 3 healthy volunteers with laying needle during venous occlusion versus without occlusion. - - - blood taking without occlusion and with the needle in place; —— blood taking after occlusion (5 min, 80 mm Hg) and with the needle in place. (healthy volunteer number 2, 3 and 4, respectively.)

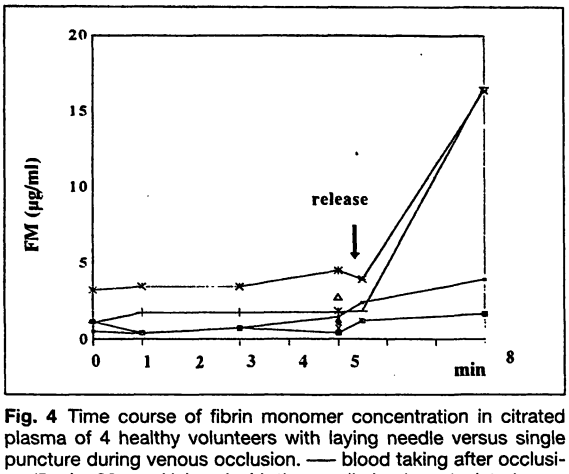

Fig. 4 Time course of fibrin monomer concentration in citrated plasma of 4 healthy volunteers with laying needle versus single puncture during venous occlusion. — blood taking after occlusion (5 min, 80 mm Hg) and with the needle in place; isolated symbols: blood taking after occlusion (5 min 80 mm Hg), single puncture. -■- ×, -+- ◆, -✳- △, -□- ⧗ healthy proband number 5, 7, 8, 9, respectively.

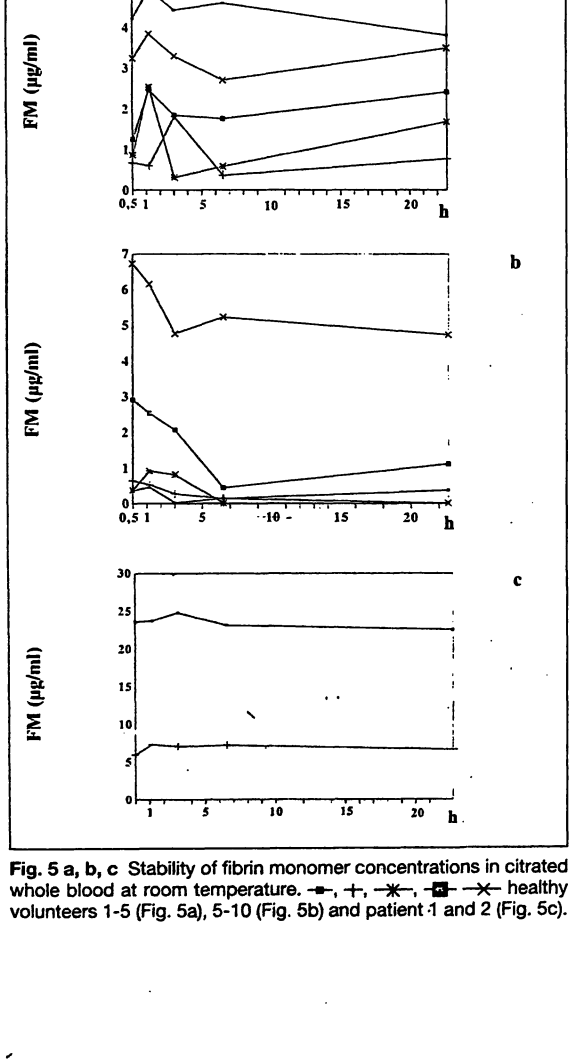

Fig. 5 a, b, c Stability of fibrin monomer concentrations in citrated whole blood at room temperature. -■-, -+-, -✳-, -□- -×- healthy volunteers 1-5 (Fig. 5a), 5-10 (Fig. 5b) and patient 1 and 2 (Fig. 5c).

Table 5 Influence of various free hemoglobin concentrations on measured fibrin monomer concentration in citrated plasma

Patient/ volunteer	Sample	FM conc. (µg/ml)	Free hemoglobin conc. (mg/dl)
Volunteer A	Native		0.2
	+ 5 µl	3.3	221.0
	+ 6.5 µl	3.8	281.0
	+ 9 µl	4.6	378.9
Volunteer B	Native	0.4	0.7
	+ 5 µl	1.7	211.9
	+ 6.5 µl	2.1	282.3
	+ 9µl	3.1	389.9
Volunteer C	Native	1.9	0.6
	+ 5 µl	3.3	239.8
	+ 6.5 µl	3.7	277.9
	+ 9 µl	4.5	384.4
Patient 1	Native	34.9	2.7
	+ 5 µl	36.6	231.9
	+ 6 µl	34.8	291.8
	+ 9 µl	38.4	404.5
Patient 3	Native	37.8	0.5
	+ 5 µl	40.2	222.2
	+ 6.5 µl	39.9	284.2
	+ 9 µl	41.0	396.7
Patient 4	Native	30.3	3.7
	+ 5 µl	32.8	220.1
	+ 6.5 µl	33.3	294.3
	+ 9 µl	34.0	400.4

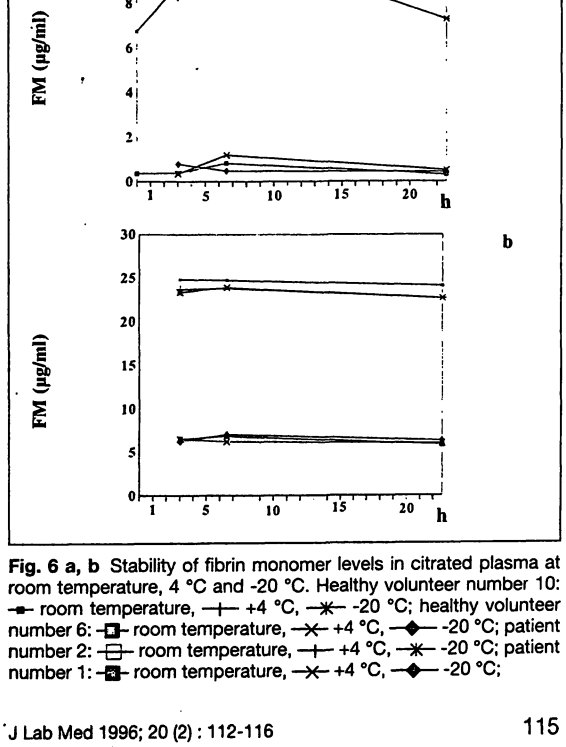

Fig. 6 a, b Stability of fibrin monomer levels in citrated plasma at room temperature, 4 °C and -20 °C. Healthy volunteer number 10: -■- room temperature, -+- +4 °C, -✳- -20 °C; healthy volunteer number 6: -□- room temperature, -×- +4 °C, -◆- -20 °C; patient number 2: -⊟- room temperature, -+- +4 °C, -✳- -20 °C; patient number 1: -▣- room temperature, -×- +4 °C, -◆- -20 °C;

shown in this study, justifies the use of ES 700 for the compilation of the blood draw and storage data.

Conclusions

According to our data, Enzymun-Test® FM has three important advantages for the every-day clinical routine:

1) Fibrin monomer values remain practically unchanged under normal blood taking conditions applying venous occlusion up to 5 minutes. Blood drawing after more than 5 minutes after setting the occlusion and also after release of the pressure should be avoided.

2) Storage of samples is possible without problems. Citrated whole blood and plasma can be stored up to one day at room temperature. In addition, freezing and thawing has not a clinically relevant effect on the FM values in citrated plasma samples.

3) No interference was observed with high bilirubin and triglyceride concentrations. The slight effect of hemolysis appears not to be clinically relevant but as a general rule hemolytic plasma samples are not regarded suitable for coagulation studies [4].

Especially with regard to the blood taking conditions FM shows superiority to other activation markers, e.g. thrombin-antithombin III complexes and prothrombin activation fragment 1+2 which are known to react sensible to changes in the conditions of blood draw [1].

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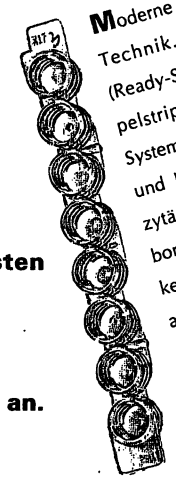

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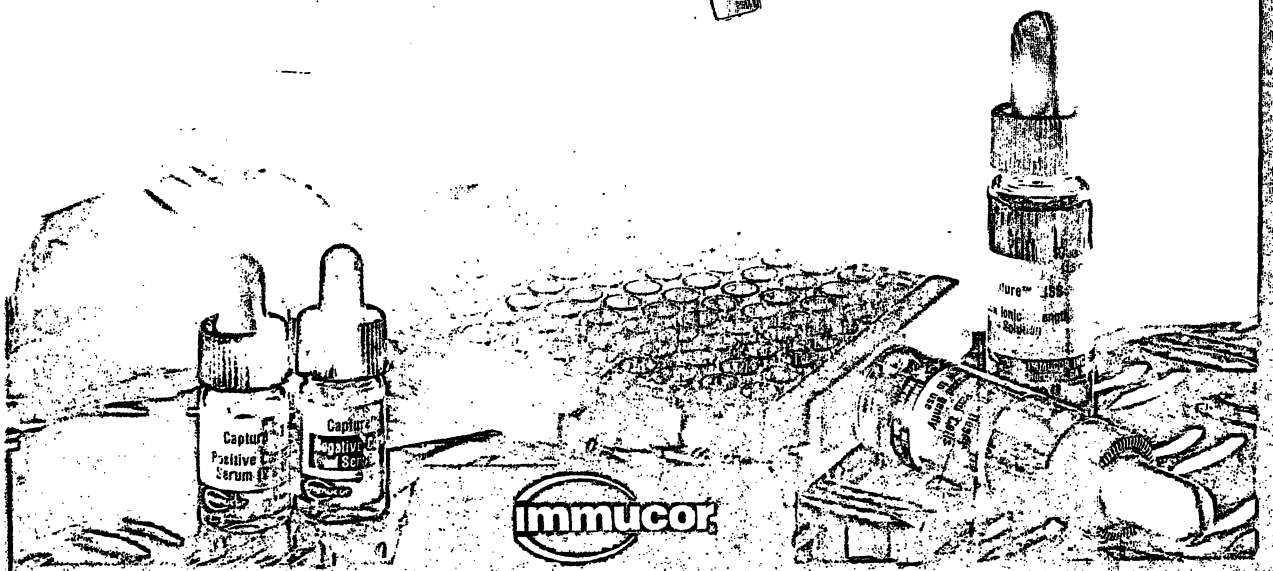

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