Comparison of counterimmunoelectrophoresis (CIE) using serum and urine specimens with culture of pernasal swabs

	No of patients	Number positive/Number tested (%)			
		CIE			Culture
		Serum	Urine	Total	Pernasal swab
Early pertussis	8	5/8 (63)	2/4 (50)	7/12 (58)	4/8 (50)
Late pertussis	9	4/9 (44)	1/5 (20)	5/14 (38)	4/9 (44)
Total	17	9/17 (53)	3/9 (33)	12/26 (46)	8/17 (47)
Control	18	0/18 (0)	0/18 (0)	0/36 (0)	ND

ND = Not done.

detecting *B pertussis* antigen and may be a useful addition in the diagnosis of whooping cough. There were no false positive reactions and no cross reactivity was found between the antipertussis antiserum and *Streptococcus pneumoniae* and *Haemophilus influenzae* antigens. The method was easy to perform and required only small quantities of specimens and laboratory reagents.

CIE was more sensitive than culture methods early in the course of the illness, when the importance of symptoms is more difficult to assess. Moreover, unlike pernasal swabs specimens of serum and urine are easy to obtain and the availability of the technique may encourage general practitioners to use this laboratory facility to complement clinical diagnosis of whooping cough.

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Requests for reprints to: Mr PC Boreland, Microbiology Department, Northern Area Laboratory, Cushendall Road, Ballymena, Co Antrim, N Ireland.

Sample collection for determination of plasma fibronectin concentration

PEARL TCY TOY, MARION E REID *Blood Bank, San Francisco General Hospital; Department of Laboratory Medicine, University of California, San Francisco; and American Red Cross, Central California Regional Blood Services, San Jose, California, USA*

Convenient blood collection methods for human plasma fibronectin determination have not been fully explored. We have investigated whether glass tubes can be used as an alternative to the recommended plastic tubes for collection¹ and whether citrated blood samples can be stored at 4°C for 22-26 h before separation and testing.

Material and methods

INTRA-ASSAY COEFFICIENT OF VARIATION
Fibronectin concentrations were determined by the

immunoturbidimetric method (Cappel, Cochranville, PA).² In order to assess the precision of this method, the intra-assay coefficient of variation was determined on two samples with different fibronectin concentrations. One observer performed 15 tests on each sample in one day, using the same dilution from an assay kit.

GLASS v PLASTIC COLLECTION TUBES

Blood was obtained from 20 volunteers, 10 men and 10 women. Blood from each donor was collected into two glass (Becton-Dickinson, Rutherford, NJ) and two plastic (polystyrene, Falcon) tubes and mixed well. Citrate anticoagulant was used in all tubes (0.5 ml of 0.129 M buffered citrate solution and 4 ml whole blood).

One glass and one plastic tube from each donor were separated by centrifugation (Sorvall CW-1) at 3400 rpm for 15 min at room temperature within 30 min of collection. Testing was performed within 4.5 h of collection. Results were analysed using the *t* test.

OVERNIGHT STORAGE AT 4°C

One plastic and one glass tube from each of the 20 donors were stored 22–26 h at 4°C before separation and testing. Values were compared with those tested within 4.5 h and analysed by the *t* test.

Results

INTRA-ASSAY COEFFICIENT OF VARIATION

The coefficient of variation on a sample with $284 \pm 7 \mu\text{g/ml}$ (mean ± 1 SD) of plasma fibronectin was 2.6%.

The coefficient of variation on a sample with a higher concentration of $439 \pm 22 \mu\text{g/ml}$ was 5.1%.

GLASS v PLASTIC TUBES

No significant difference ($p > 0.05$) was found between samples collected in glass and those collected in plastic tubes (Table).

OVERNIGHT STORAGE AT 4°C

No significant difference ($p > 0.05$) was found between samples tested after overnight storage and those tested within 4.5 h of collection (Table).

Discussion

This study has shown that blood for plasma fibronectin determination may be collected in glass or plastic tubes with citrate anticoagulant, and that samples may be stored overnight at 4°C before separation and testing by the immunoturbidimetric method.

Previous studies have shown that plasma fibronectin

Plasma fibronectin concentrations in 20 normal donors

Plasma fibronectin concentration ($\mu\text{g/ml}$)	Time tested after collection			
	<4.5 h		24 h	
	Glass	Plastic	Glass	Plastic
Mean	317	316	318	313
SD	49	48	47	48

Blood from each donor was collected in citrated glass and plastic tubes and tested within 4.5 h and after overnight storage at 4°C.

tin concentrations are affected by the anticoagulant used. Heparin is an unacceptable anticoagulant because fibronectin concentrations are reduced after storage for six days to five weeks.^{1,2} EDTA is acceptable for six days storage at 25 or -16°C if aprotinin is added,² but considerable fluctuations in concentrations occur after freeze thawing.¹ Citrate is the best anticoagulant for longer (five weeks) frozen storage.¹ The present study showed that citrate was acceptable for overnight 4°C whole blood sample storage as well. Citrate may therefore be the best anticoagulant for both liquid and frozen storage of plasma samples.

Plastic tubes have been recommended for collection.¹ This study suggests that fibronectin adherence to glass tubes does not result in a significant decrease in plasma concentrations during overnight storage. The presence of other proteins such as albumin may decrease adherence of fibronectin to surfaces.³

Liquid storage of plasma for no more than 3 h has been recommended because plasma proteases may breakdown fibronectin.¹ This study suggests that proteolytic activity does not affect fibronectin concentrations during overnight storage of whole blood samples. Addition of protease inhibitors has been suggested for liquid storage of patient samples up to six days.²

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Requests for reprints to: Dr Pearl Toy, Chief, Blood Bank, San Francisco General Hospital, 1001 Potrero Ave, San Francisco, California, 94110, USA.