

# Comparison of platelet storage in PL146 and PL732 plastic packs: preliminary in vitro studies

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**SUMMARY** This paper is a preliminary report of in vitro studies comparing platelet storage in the new polyolefin PL732 packs with the present polyvinyl PL146 packs. The parameters used to assess platelet viability in these studies were the recovery from the hypotonic stress test (HST) and the pH. The effect of the mass of the concentrate was assessed by preparing 20 g, 35 g and 50 g packs. The method of agitation was tested comparing a vertical rotator with a flat-bed, rocking agitator.

In all the subgroups the PL732 packs fared better than the PL146 packs in terms of HST recovery and pH. The 35 g and 50 g packs were statistically inseparable but the 20 g packs were significantly worse both in PL732 and PL146. The vertical rotator augmented the beneficial effects of the PL732 plastic especially in terms of pH. The 35 g and 50 g packs in PL732 on a vertical rotator gave results after 5 days storage at 22°C which were vastly superior ( $p \leq 0.001$ ) to the 35 g and 50 g packs in PL146 after only three days storage. Thus it would appear that the use of PL 732 packs might enable the storage life of platelet concentrates to be increased to five days. Further in vivo studies are to be undertaken to ensure these in vitro improvements benefit the patient.

Platelet concentrates stored in conventional polyvinyl packs (PL146 Travenol), have a shelf-life of two to three days at 22°C. After this time the accumulation of CO<sub>2</sub> and lactate cause the pH to fall below 6.0 with disastrous effects on platelet viability.<sup>1-4</sup> Improvement of gaseous exchange between the concentrates and the atmosphere should delay accumulation of CO<sub>2</sub> and prolong storage life. Garber described a polyolefin pack (PL732 Travenol) which showed improved gaseous permeability when compared with the PL146 pack<sup>5</sup> and work by Murphy and Holme<sup>6</sup> suggested improved platelet viability both in vitro and in vivo.

To substantiate these suggestions a three-centre trial was begun involving the Transfusion Centres of Dublin, Manchester and Oxford, each working independently. The first stage of this trial was to confirm the in vitro characteristics of the PL732 packs. This paper reports the findings at the Oxford Centre.

## Material and methods

Whole blood was drawn from 84 healthy donors who had not taken aspirin in the past seven days. Care was taken to ensure a clean venepuncture. All packs were

supplied by Travenol Laboratories Ltd, Thetford, Norfolk. Triple packs were used, the primary pack being of PL146 and containing CPD anticoagulant. Forty-two packs had conventional satellites of PL146 plastic and 42 had satellites of PL732 for the storage of platelets. All concentrates were prepared at room temperature within two hours of collection.

The initial centrifugation to prepare platelet-rich plasma (PRP) was at 1000 g for 7 min. A weighed 180 g of PRP was transferred to the satellite pack giving a mean yield of  $79 \pm 18\%$  of the available platelets. A second centrifugation at 2100 g for 20 min produced platelet-poor plasma (PPP) and a button of platelets. Weighed amounts of PPP were transferred to the second satellite using a plasma-extractor (Fenwal BM1) and a spring-balance (Salter) to leave 20 g, 35 g and 50 g amounts in which to resuspend the platelets. The weights of the concentrates were subsequently checked and all the packs were found to be within 3 g of their desired weight. The platelets were left undisturbed at room temperature for at least 90 min before re-suspension.

## STORAGE

All concentrates were stored in a 22°C incubator (Termacks). Because of reports that the method of agitation might influence viability<sup>7</sup> (also S Murphy, personal communication, 1981), equal numbers of

packs were stored on either: (i) a vertical rotator (Helmer PA4) with each pack separate and turning at 5 rpm or (ii) a rocking flat-bed agitator (Luckhams) in batches of 4-6 and moving at 25 oscillations/min.

#### DETERMINATION OF pH

This was measured on the pH electrode of an ABL2 blood-gas analyser.

#### DETERMINATION OF CONCENTRATION OF PLATELETS

This was derived from the platelet count of a Coulter S Plus II.

#### DETERMINATION OF HYPOTONIC STRESS TEST (HST)

The method described by Handin *et al*<sup>8</sup> was modified as follows: a Pye Unicam SP 8100 recording spectrophotometer was used with the wavelength set at 610  $\mu\text{m}$  and zeroed for absorbance on a cuvette of distilled water. Full-scale deflection was set at 0.500 optical density units (ODU) and the trace recorded at 100 s/cm. The distilled water was used as a blank in the second channel during the tests: 0.3 ml of distilled water was rapidly added to 0.6 ml of platelet concentrate and the changes in optical density in ODU were recorded for the next 5 min. The usual pattern was a rapid fall in optical density followed by a recovery. The degree of recovery has been shown to be a function of platelet viability.<sup>9</sup>

#### SAMPLING

All samples from a given pack were taken through a self-sealing entry port (Travenol C2405). Care was

taken to avoid contamination and all packs were subsequently shown to be sterile after five days by routine bacteriological plating and culture. Sample size was kept to 1.2 ml in order to avoid excessive loss of platelets during the experiments.

#### Results

The 84 packs were tested under varying conditions of pack material, method of agitation and weight of concentrate. This gave rise to 12 subgroups with seven samples in each. Occasional concentrates were found to have very high or very low yields which were atypical for their group and on testing gave results that were outside the 95% confidence limits. Five such outliers were excluded from further analysis and none of the 12 groups contained more than one outlier.

#### HYPNOTIC STRESS TEST (HST)

Preliminary experiments showed that the recovery from the lowest recorded optical density to the five-minute point was reproducible to within  $\pm 0.001$  ODU for a given pack on a given day. As this series compares packs with different concentrations it was decided to follow the practice of Kim and Baldini<sup>9</sup> calling the recovery in ODU on a day nought 100% and expressing subsequent results as a percentage of this value. Figs 1 and 2 show little difference between the three weights of concentrate in PL146 whichever method of agitation is used. The 20 g concentrates in PL732 show a similar pattern but the 35 g and 50 g packs in PL732 show a marked improvement in recovery both on day three and day five. Analysis of

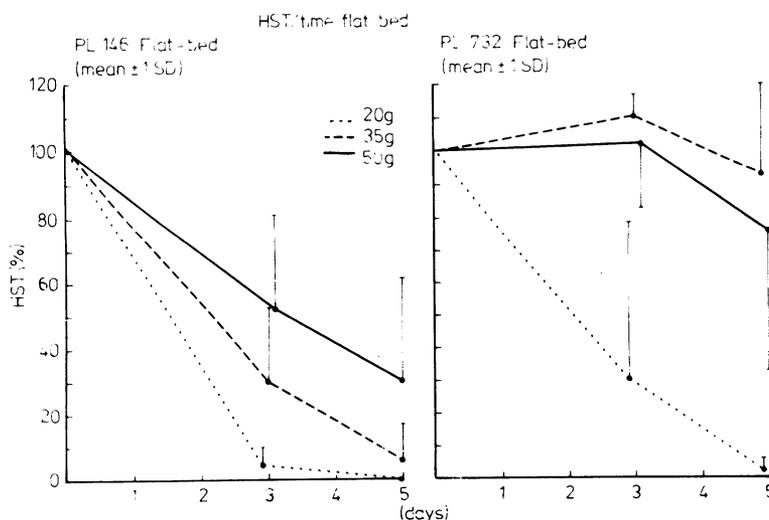


Fig. 1 Hypotonic stress recovery (mean  $\pm$  1SD) of packs in PL146 and PL732 on a Flat-Bed Agitator

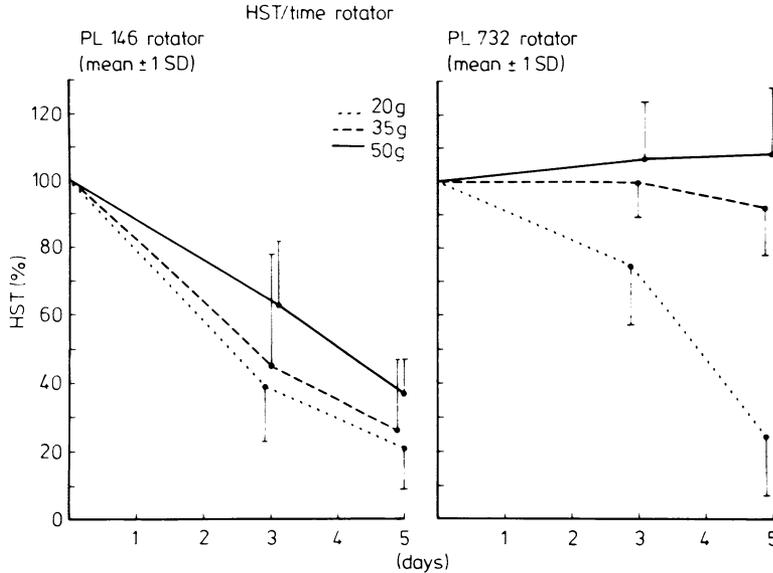


Fig. 2 Hypotonic stress recovery (mean ± 1SD) of packs in PL146 and PL732 on a Rotator

the 35 g and 50 g packs as a whole (Fig 3 and Table 1) reveals a significantly better recovery of platelets in PL732 both on days three and five compared to the day three recovery in PL146. Statistically p is always <0.01 and often <0.001. The difference between the day five values in PL732 was uninfluenced by the method of agitation ( $p > 0.1$ ).

**pH**  
The pH shows a broadly similar pattern to the HST

results. Again there is little difference between the three PL146 concentrates and the 20 g PL732 concentrates but the 35 g and 50 g PL732 concentrates show improved maintenance of pH. This difference is more marked when the platelets are stored on a rotator (Figs. 4, 5, 6 and Table 2). Under these circumstances the 35 g and 50 g packs show a rise in pH on day three.

**Comment**

The in vitro parameters used in this study show that PL732 is significantly better than PL146 for storing platelets, provided there is an adequate volume of concentrate. The 20 g pack of PL732 fared no better

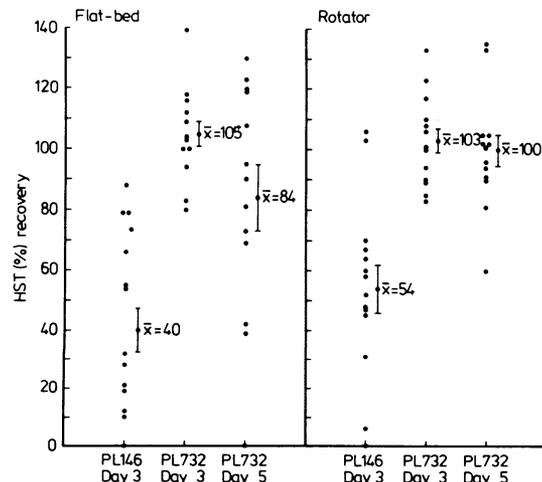


Fig. 3 Pooled results of HST recovery in 35 g and 50 g packs (mean ± 1SE) comparing day 3 in PL146 with days 3 and 5 in PL732 on both Flat-Bed and Rotator

Table 1 Values for p derived from Fig. 3 showing the difference between day 3 in PL146 and days 3 and 5 in PL732 in terms of HST recovery. (Pooled 35 g and 50 g packs)

Flat-Bed			
	PL146	PL732	p
Day 3		3	≤0.001
Day 3		5	≤0.01
Rotator			
Day 3		3	≤0.001
Day 3		5	≤0.001

PL146 FB Day 3: PL732 Rotator Day 5  $p \leq 0.001$   
 PL732 FB Day 5: PL732 Rotator Day 5  $p > 0.1$

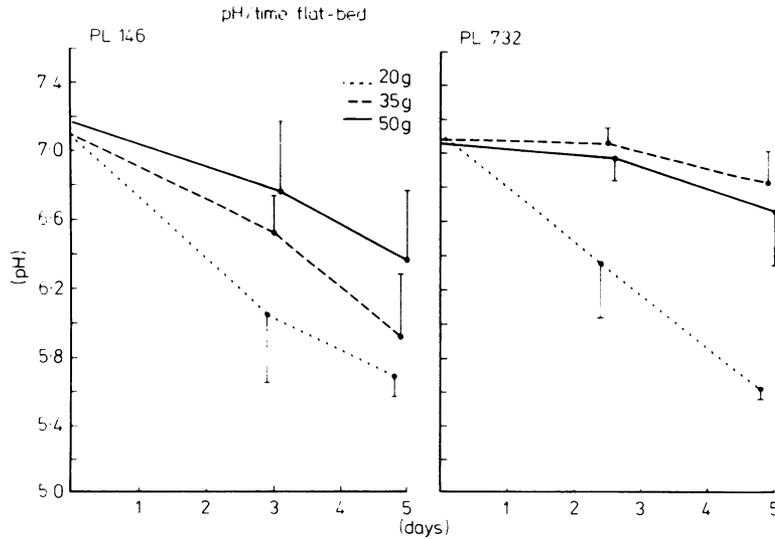


Fig. 4 pH change (mean  $\pm$  1SD) of packs in PL146 and PL732 on a Flat-Bed Agitator

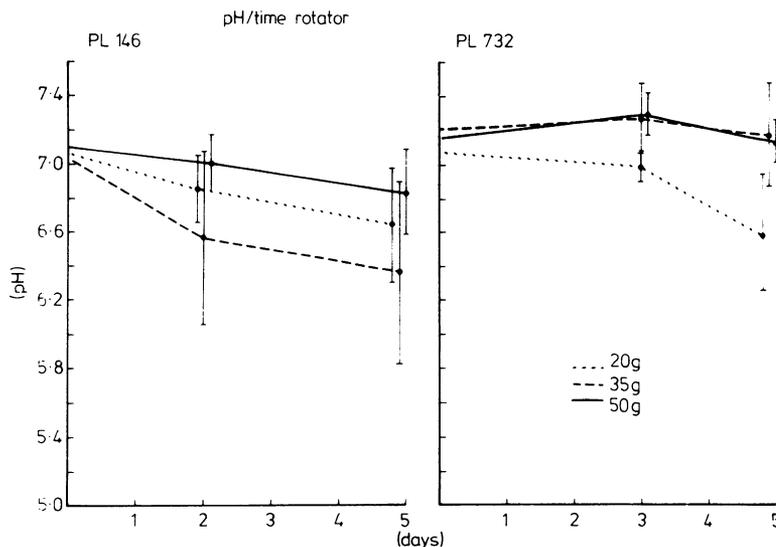


Fig. 5 pH change (mean  $\pm$  1SD) of packs in PL146 and PL732 on a Rotator

than the PL146 packs and in general the 20 g packs were useless by day five. In marked contrast the 35 g and 50 g concentrates in PL732 were significantly better than their counterparts in PL146, whether on a flat-bed or a rotator.

The improvement was most marked in terms of the HST. Platelet response in the HST was shown to correlate with the *in vivo* post-transfusion recovery by Valerie *et al*<sup>10</sup> and with viability by Kim and Baldini.<sup>9</sup> A combination of PL732 and rotator gave

recoveries at day five which were a little different from day 0 values and very much better ( $p < 0.001$ ) than the day three recoveries in PL146.

The differences in pH were not so well marked and indeed the day five values in PL732 on a flat-bed were not significantly better than the day three values in PL146. The rotator maintained pH better than the flat-bed in all groups, though the improvement was most marked in the PL732 packs. The day five values in PL732 on a rotator were much better than the day

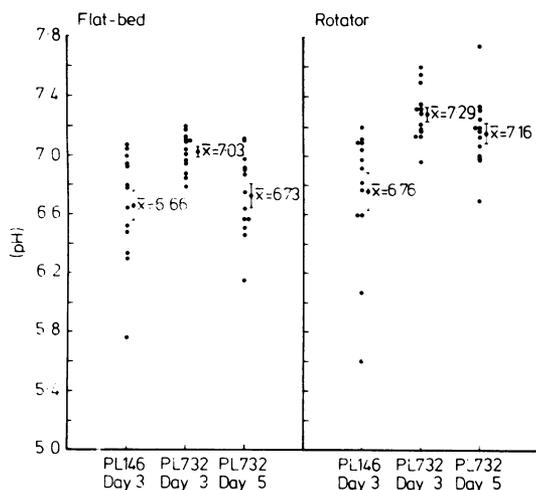


Fig. 6 Pooled results of pH values in 35 g and 50 g packs (mean  $\pm$  ISE) comparing day 3 in PL146 with days 3 and 5 in PL732 on both Flat-Bed and Rotator

Table 2 Values for p derived from Fig. 6 showing the difference between day 3 in PL146 and days 3 and 5 in PL732 in terms of pH (pooled 35 g and 50 g packs)

Flat-Bed			
	PL146	PL732	p
Day	3	3	$\leq 0.002$
Day	3	5	$> 0.1$ (NS)
Rotator			
Day	3	3	$\leq 0.001$
Day	3	5	$\leq 0.01$

PL146 FB Day 3: PL732 Rotator Day 5  $p \leq 0.001$   
 PL732 FB Day 5: PL732 Rotator Day 5  $p > 0.1$

five values in PL732 on a flat-bed ( $p < 0.001$ ). Thus it would appear that the rotator helps to maintain the pH, but this is not the sole reason for the improvement in HST seen in the PL732 packs. Further studies looking at the platelet morphology and gaseous exchange will be reported in subsequent papers.

Obviously in vivo studies are necessary to show that these in vitro improvements benefit the patient. These form the basis of the later part of this trial and

will be reported in due course. With the parameters so far investigated it would appear that five days storage in PL732 is less detrimental to platelets than three days in a conventional PL146 pack. The use of the rotating agitator gives the additional benefit of a more physiological pH for transfusion. However both these benefits may be lost if the volume of concentrate is too small. Regulation of volume to about 40 ml seems sensible and a calculation of the weight of pack contents is an easy way to achieve this.

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