

A multicenter inspection of the swirling phenomenon in platelet concentrates prepared in routine practice

F. BERTOLINI AND S. MURPHY FOR THE BIOMEDICAL EXCELLENCE FOR SAFER TRANSFUSION (BEST) WORKING PARTY OF THE INTERNATIONAL SOCIETY OF BLOOD TRANSFUSION

Background: In a previous multicenter study, the absence of the swirling phenomenon correlated well with platelet disc-to-sphere transformation, a morphology feature associated in the past with poor platelet viability.

Study Design and Methods: In 13 centers, 5366 platelet concentrates (PCs) were prepared and stored under routine conditions and evaluated for the presence of swirling. PCs found not to have swirling were evaluated for pH and platelet and white cell counts in parallel with an equal number of PCs with swirling that were studied as controls.

Results: In 13 participating centers, swirling was reported to be absent after 3 and 5 days of storage in 0 to 3.8 percent and in 0 to 18 percent of PCs, respectively. In 94 percent of units with swirling, pH was 6.7 to 7.5; this is a range of values associated with adequate in vivo survival. In 69 percent of PCs without swirling, pH was lower than 6.4 or higher than 7.6; these values are associated with reduced platelet viability. Three-quarters of the cases in which the absence of swirling was associated with pH values between 6.7 and 7.5 were reported from 2 out of the 13 participating centers.

Conclusion: In PCs prepared and stored under routine conditions, the presence of swirling correlated well with pH values associated with adequate platelet in vivo viability. In about 20 percent of PCs without swirling, this was not due to a low or high pH value, and it would be useful to investigate whether this lack of swirling is associated with poor platelet viability. These data support the possibility that the evaluation of swirling by visual inspection might replace invasive and more cumbersome assays currently used for routine PC quality control.

Abbreviations: BC = buffy coat; PC(s) = platelet concentrate(s); PRP = platelet-rich plasma.

QUALITY CONTROL OF platelet concentrates (PCs) includes invasive evaluations such as monitoring of pH and of platelet number, size, morphology, and response to hypotonic stress and aggregating agents.¹ Platelet discoid morphology is known to correlate well with the in vivo viability of platelets, and factors affecting discoid morphology (pH drop to 6.7 or less, exposure to cold, storage lesion) are also known to affect platelet viability.²⁻⁹ Discoid platelets, exposed to a light source, reflect that light, producing the "swirling" phenomenon that can be easily evaluated by examining a PC against a light source while gently rotating the container or gently squeezing the PC with one thumb. In a previous article,¹⁰ we described a multicenter evaluation of the reproducibility of swirling in PCs and concluded that the inspection of swirling could be a promising tool for quality control. In

fact, PCs containing spherical platelets, obtained by adding refrigerated platelets to fresh PCs, were reported to have extensive swirling (i.e., they were said to be swirling-positive), some swirling (swirling-intermediate), and no swirling (swirling-negative) in 5, 27, and 68 percent of cases, respectively. When standard PCs were examined, positive, intermediate, and negative swirling was shown in 83, 15, and 2 percent of 1-day-old PCs versus 65, 24, and 11 percent of 5-day-old PCs, respectively. Results in PCs containing a known proportion of spherical (cold) platelets were intermediate between those in completely spherical and regular, fresh PCs. In these studies, most PCs containing 20 mL of regular platelets and 40 mL of spherical platelets were reported to have intermediate swirling, whereas most PCs containing 40 mL of regular platelets and 20 mL of spherical platelets were reported to be swirling-positive. Moreover, concordance in the swirling evaluation performed by two technicians was good.

Notwithstanding these good premises, data collected in the previous study were not representative of actual routine conditions, as all PCs evaluated were selected to

From the Centro Trasfusionale e di Immunologie dei Trapianti, Ospedale Maggiore Policlinico, Milan, Italy; and the American Red Cross Blood Services, Penn-Jersey Region, Philadelphia, Pennsylvania.

Received for publication March 2, 1995; revision received and accepted September 8, 1995.

TRANSFUSION 1996;36:128-132.

have a pH ranging from 6.8 to 7.6 and to be free of aggregates, lipemia, or reddish plasma. Moreover, data were collected by inspection of PCs prepared by only one of the different methods currently used for PC preparation, that is, by using platelet-rich-plasma (PRP). The present study was aimed at investigating in routine practice the percentage of nonswirling PCs stored in blood centers of different countries and at evaluating whether a drop in pH is the main reason for the absence of swirling. Accumulation of lactate due to glycolysis occurs at a rate of 2.5 mM per day in PCs, but lactate production is six to seven times higher when oxygen supply is not adequate.⁹ Hydrogen ions generated by this source can reduce the pH of PCs to values below 6.8 and can cause platelet swelling⁷ and loss of discoid shape.⁸ When the pH ranges from 6.4 to 6.7, these changes seem to be reversible, but when the pH falls below 6.1 to 6.3, a return to the original shape is not possible.^{11,12} In this context, we were interested in evaluating in routine practice whether swirling was present in PCs with pH values in the range correlating with good viability (6.7-7.5), moderate platelet lesion (6.4-6.6), or severe (<6.3) platelet lesion. In the previous study of the Biomedical Excellence for Safer Transfusion (BEST) Working Party of the International Society of Blood Transfusion,¹⁰ swirling was scored as extensive (positive), moderate (intermediate), or absent (negative), and it was correlated with platelet morphology. This study was aimed at monitoring swirling as a marker of an adequate pH value, and swirling was reported only as present (positive) or absent (negative). Data were collected by monitoring different types of PCs, including single- and multiple-donor PCs and PRP- and buffy coat (BC)-derived multiple-donor PCs.

Materials and Methods

A total of 13 centers participated in the study, and principal investigators are listed in Table 1.

Training for swirling evaluation

In centers without extensive experience in swirling evaluation, the staff in charge of this inspection was trained by evaluating swirling in PCs exposed to cold (swirling-negative control) and in 1-day-old PCs (swirling-positive control). Fresh PCs used as negative controls were stored at 4°C for 18 to 24 hours, stored at 22°C for 1 hour without agitation, resuspended at 22°C in an agitator for 1 hour, and checked for complete resuspension. Technician(s) in training evaluated swirling by examining the PC bag against a 50- to 100-W white light at a distance of 30 to 70 cm from the light. If there was swirling, it was obtained by gently rotating the bag or gently squeezing it with one thumb. Each technician in charge of swirling evaluation determined swirling in three negative controls and in three positive controls (1-day-old, routinely prepared PCs) for 5 consecutive days over a 2-week period.

Swirling evaluation

After the training period, technicians evaluated swirling, as reported above, in routinely produced PCs. Different types of

Table 1. Principal investigators (in alphabetical order)

Principal investigator	Center
M.A. Blajchman	McMaster University Medical Center, Hamilton, ON, Canada
D. Ciavarella	Hudson Valley Blood Service, Valhalla, NY
T.J. Greenwalt	Hoxworth Blood Center, Cincinnati, OH
C. Höglman	University Hospital, Uppsala, Sweden
M. Masse	Centre Régional de Transfusion Sanguine, Besançon, France
L. Messeter	University Hospital, Lund, Sweden
N. Müller	University Hospital, Essen, Germany
S. Murphy	Penn-Jersey Red Cross, Philadelphia, PA
G. Myllylä	Finnish Red Cross, Helsinki, Finland
R.N.I. Pietersz	Red Cross Blood Bank, Amsterdam, the Netherlands
S. Sekiguchi	Hokkaido Red Cross Blood Center, Sapporo, Japan
G. Sirchia	Centro Trasfusionale e di Immunologia dei Trapianti, Milan, Italy
C.Th. Smit Sibinga	Red Cross Blood Bank, Groningen, the Netherlands

PCs suitable for the study included random-donor, PRP-derived PCs (volume, 40-70 mL); random-donor, BC-derived PCs (volume, 40-70 mL); random-donor, PRP-derived PCs stored as a pool (volume, 300-400 mL); random-donor, BC-derived PCs prepared and stored as a pool (volume, 300-400 mL); and single-donor apheresis PCs (volume, 300-500 mL). PCs were stored at 22°C in a tumbler rotator or a reciprocator. Swirling was evaluated on Day 3 of storage (i.e., 49-72 hours after blood donation), and the result was reported as positive or negative. When a PC was found to be swirling-negative, a sterile PC sample of 2 to 3 mL was obtained for automated measurement of platelet and white cell counts and pH at 22°C. If pH was determined at 37°C, the 22°C value was computed by the following formula:

$$\text{pH at } 22^{\circ}\text{C} = \text{pH at } 37^{\circ}\text{C} + 0.22$$

Platelet and white cell automated counts and pH at 22°C were also determined in a similar number of positive controls. For every negative PC, the positive PC that immediately followed in the daily routine production was evaluated. If the PC evaluated on Day 3 was still available in the blood center on Day 5 of storage (i.e., 97-120 hours after blood donation), swirling was evaluated again. For each PC evaluated on Day 5, a 5-day-old swirling-positive PC was monitored as a control.

Statistical analysis

We performed statistical analysis by using statistical analysis software (SAS Institute, Cary, NC). The Wilcoxon's signed rank sum test was used to evaluate differences in the platelet and white cell counts and pH values in swirling-positive and swirling-negative PCs.¹³

Results

A total of 5366 PCs were evaluated on Day 3, and 2751 of the same series of PCs were evaluated on Day 5. As reported in Table 2, which also lists centers where technologists received the specific training in swirling evaluation described in Materials and Methods, 0 to 3.8 percent of PCs evaluated on Day 3 of storage were reported as swirling-negative, while 0 to 18 percent of PCs evaluated on Day 5 of storage were reported as swirling-negative. Overall, no correlation was found between the presence or absence of swirling and platelet or white cell

Table 2. The pH values and percentage of swirling-positive ($n = 132$) and swirling-negative ($n = 146$) PCs

Center number*	PC type	Day	Number	Number (%) swirling negative	pH	
					Swirling-negative control	Swirling-positive control
1†	PRP-PC	3	522	0		
		5	46	2 (4.3)	7.24 ± 0.1	7.28 ± 0.12
2	PRP-PC	3	599	9 (1.5)	6.4 ± 0.6	7.3 ± 0.1
		5	106	15 (14)	6.2 ± 0.4	7.1 ± 0.2
3†	PRP-PC	3	942	5 (0.5)	6.56 ± 0.89	7.38 ± 0.04
		5	493	9 (1.9)	5.97 ± 0.92	7.28 ± 0.20
4	PRP-PC	3	621	1 (0.16)	7.15	7.20
		5	231	2 (0.86)	6.65 ± 0.36	6.92 ± 0.03
5	PRP-PC	3	522	8 (1.5)	7.93 ± 0.26	7.68 ± 0.23
		5	522	17 (3.2)	7.17 ± 0.93	7.44 ± 0.31
6†	PRP-PC	3	400	0		
		5	400	2 (0.5)	5.9 ± 0.1	7.1 ± 0.1
7	BC-PC	3	332	5 (1.5)	7.35 ± 0.05	7.37 ± 0.08
	(random)	5	405	8 (1.9)	7.03 ± 0.31	7.33 ± 0.13
8	BC-PC	3	569	3 (0.5)	6.8 ± 0.6	7.0 ± 0.1
	(random)	5	153	28 (18)	7.00 ± 0.35	7.05 ± 0.18
9	BC-PC	3	106	1 (0.9)	6.4	6.7
	(pool)	5	174	16 (9.1)	6.37 ± 0.08	6.52 ± 0.14
10	BC-PC	3	105	1 (0.9)	6.25	6.55
	(pool)	5	52	3 (5.7)	6.42 ± 0.02	6.84 ± 0.01
11	BC-PC	3	194	0		
	(pool)	5	67	3 (4.4)	6.3 ± 0.1	7.4 ± 0.2
12	Apheresis	3	77	3 (3.8)	6.03 ± 0.20	6.8
		5	7	0		
13	Apheresis	3	305	0		
		5	76	0		
8	Apheresis	3	71	0		
		5	29	5 (17)	6.58 ± 0.29	7.08 ± 0.08

* Not correlated with the alphabetical order of investigators in Table 1.

† Centers where specific training in swirling evaluation was conducted because of limited or no experience with the method.

count. In Center 5, the pH was found to be slightly, albeit not significantly, higher than that in other centers. It is interesting that, in that center, there was a significant difference in the platelet counts in the swirling-negative PRP-PCs ($45 \pm 24 \times 10^9$ platelets) and the swirling-positive PRP-PCs ($82 \pm 35 \times 10^9$

platelets). The pH of PCs reported as swirling-negative or -positive is shown in Fig. 1, which presents data from the 13 centers participating in the study.

The number of swirling-negative PCs evaluated (146) was slightly higher than the number of swirling-positive control PCs

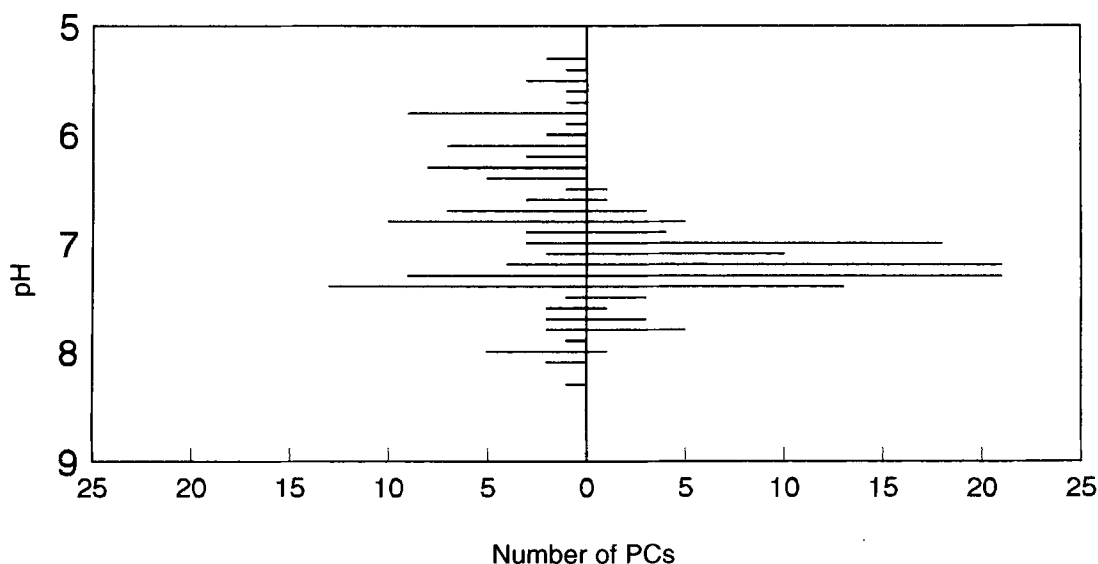


FIG. 1. Distribution of pH values in PCs reported as swirling-negative (left) or swirling-positive (right) in 13 centers participating in the study. The number of swirling-negative PCs evaluated (146) was slightly higher than the number of swirling-positive control PCs evaluated (132), because in some centers 5-day-old swirling-positive BC-PCs or apheresis PCs were not always available. The pH of swirling-negative PCs differs significantly from that of swirling-positive PCs ($p < 0.001$). However, pH values in swirling-negative PCs were more scattered than those in swirling-positive PCs.

evaluated (132), because in some centers a 5-day-old swirling-positive BC-PC or apheresis PC was not always available. The presence of swirling was associated in 94 percent of cases with a pH value in the range of 6.7 to 7.5; this is a range of values associated with adequate *in vivo* survival.^{2,3,5-9} Although data from Center 9 indicated that the pH in swirling-negative PCs was significantly lower than that in swirling-positive PCs (6.37 ± 0.08 vs. 6.52 ± 0.14), these 17 outliers were not presented in Fig. 1. In fact, these BC-PCs were stored in a synthetic medium lacking a buffer for lactic acid. At Center 9, *in vitro* measurements reflecting platelet morphology, metabolism, and function (possibly correlating with swirling) were still under evaluation. The pH values of PCs reported as swirling-negative were more scattered than those of swirling-positive PCs. However, the pH values in swirling-negative PCs differed significantly from those in swirling-positive PCs ($p < 0.001$). It could be noted from Fig. 1 that 48 percent of swirling-negative PCs had a pH lower than 6.4 or higher than 7.5; these values are associated with poor platelet viability. However, it must be considered that 74 percent of PCs reported as swirling-negative and found to have an adequate pH (in the range of 6.7-7.5) came from just 2 of the 13 participating centers. Isolated data from these two centers indicate that swirling-positive and swirling-negative PCs had a similar pH (7.0 ± 0.2). If data from these two centers were removed from the cumulative data analysis, the pH would be 6.7 to 7.5 in less than 21 percent of swirling-negative PCs.

Discussion

In this multicenter study, we determined pH values in PCs prepared and stored in routine conditions in 13 centers in nine different countries. More than 5000 PCs were evaluated for the presence of swirling. In 132 swirling-positive PCs, the pH was measured, and, in less than 6 percent of those PCs, it was in a range associated with poor *in vivo* viability of platelets. These data indicate that the presence of swirling in PCs is informative, because it is highly effective in predicting a pH value in an adequate range. The absence of swirling was apparently less informative, because more than one-third of the PCs reported as swirling-negative had an adequate pH. However, 2 of the 13 participating centers reported 74 percent of the cases in which the absence of swirling failed to identify a PC with a pH value correlated to poor platelet viability. This suggests that training of personnel in swirling evaluation could be critical to the correct identification of swirling-negative units. This training could be done with PCs containing known proportions of spherical platelets obtained by the addition of refrigerated platelets to fresh PCs.¹⁰ In this regard, it should be mentioned that a number of devices for the automated evaluation of swirling have been proposed,¹⁴⁻¹⁷ but these instruments have not been extensively studied in routine practice.

As has been demonstrated in the previous study,¹⁰ the evaluation of swirling offers some advantages over other measurements of platelet quality and has promise for replacing invasive and more cumbersome assays in large-scale PC quality control. Evaluation of swirling is a simple and reproducible measurement reflecting platelet discoid morphology: it does not require invasive sampling

procedures or dedicated devices, and PCs can easily be checked for the presence of swirling before they are pooled or issued to the clinical ward. Moreover, other studies including *in vivo* data obtained by the transfusion of autologous PRP-PCs showed 1) that the absence of swirling correlates with a drop in pH, poor morphology, reduced hypotonic shock response, and reduced shape change with ADP and 2) that swirling-negative PCs with a good pH had less *in vivo* viability than did swirling-positive PCs with pH values in the same range¹⁸ (Holme et al., unpublished observations, 1994).

In the present study, in at least 20 percent of swirling-negative PCs, the absence of swirling was not due to a low or high pH. Whether this lack of swirling is associated with poor platelet viability in platelet recipients remains to be demonstrated. Future investigations should include *in vivo* evaluation of swirling-positive and swirling-negative PCs. In the meantime, because previous and current¹⁰ studies indicate a correlation between lack of swirling and falling pH or increasing spherical morphology, and because swirling could be absent in PCs exposed to cold or inadequate or inappropriate agitation,¹⁸ we suggest the use of swirling inspection as first-line, non-invasive PC quality control. In this context, swirling-negative PCs should be investigated further for pH and discarded if they fail to meet the current standard of pH > 6.0 . In the present study, 2.7 percent of 5366 evaluated PCs were found to be swirling-negative, and more than 12 percent of these swirling-negative PCs had pH < 6.0 , while none of the swirling-positive PCs had pH values lower than 6.4.

References

1. Snyder E. Activation during preparation and storage of platelet concentrates (editorial). *Transfusion* 1992;32:500-2.
2. Holme S, Vaidja K, Murphy S. Platelet storage at 22°C: effect of type of agitation on morphology, viability, and function *in vitro*. *Blood* 1978;52:425-35.
3. Murphy S, Gardner FH. Platelet preservation. Effect of storage temperature on maintenance of platelet viability—deleterious effect of refrigerated storage. *N Engl J Med* 1969;280:1094-8.
4. White JG, Krivit W. Ultrastructural basis for shape change induced in platelets by chilling. *Blood* 1967;30:625-35.
5. Murphy S, Sayar SN, Gardner FH. Storage of platelet concentrates at 22°C. *Blood* 1970;35:549-57.
6. Lindberg JE, Slichter SJ, Murphy S, et al. *In vitro* function and *in vivo* viability of stored platelet concentrates. Effect of a secondary plasticizer component of PVC storage bags. *Transfusion* 1983;23:294-9.
7. Holme S, Murphy S. Platelet storage at 22°C for transfusion: interrelationship of platelet density and size, medium pH, and viability after *in vivo* infusion. *J Lab Clin Med* 1983;101:161-74.
8. Holme S, Murphy S. Quantitative measurements of platelet shape by light transmission studies: application to storage of platelets for transfusion. *J Lab Clin Med* 1978;92:53-64.
9. Murphy S, Kahn RA, Holme S, et al. Improved storage of platelets for transfusion in a new container. *Blood* 1982;60:194-200.
10. Bertolini F, Murphy S. A multicenter evaluation of reproducibility of swirling in platelet concentrates. Biomedical Excellence for Safer Transfusion (BEST) Working Party of the International Society of Blood Transfusion. *Transfusion* 1994;34:796-801.

11. Solberg C, Holme S, Little C. Morphological changes associated with pH changes during storage of platelet concentrates in first-generation 3-day container. *Vox Sang* 1986;50:71-7.
12. Bertolini F, Porretti L, Lauri E, et al. Role of lactate in platelet storage lesion. *Vox Sang* 1993;65:194-8.
13. Glantz SA. *Primer of biostatistics*. New York: McGraw Hill, 1981.
14. Fratantoni JC, Poindexter BJ, Bonner RF. Quantitative assessment of platelet morphology by light scattering: a potential method for the evaluation of platelets for transfusion. *J Lab Clin Med* 1984;103:620-31.
15. Bellhouse EL, Inskip MJ, Davis JG, Entwistle CC. Pre-transfusion non-invasive quality assessment of stored platelet concentrates. *Br J Haematol* 1987;66:503-8.
16. Jaremo P, Edlund B, Berseus O, Kutti J. Some relations between light transmission changes and biochemical parameters of stored platelet packs. *Thromb Haemost* 1993;70:527-30.
17. George V, Holme S, Moroff G. Evaluation of two instruments for noninvasive platelet concentrate quality assessment. *Transfusion* 1989;29:273-5.
18. Ross D, Holme S, Hartman P, et al. A quick visual method for quality control of platelet concentrates (abstract). *Transfusion* 1986;26(Suppl):550.

Francesco Bertolini, MD, PhD, Assistant, Centro Trasfusionale e di Immunologia dei Trapianti, Department of Hematology, Ospedale Maggiore Policlinico, Milan, Italy; current address: Associate Member, Division of Medical Oncology, IRCCS Maugeri Foundation, Pavia Medical Center, viale Boezio 26, 27100 Pavia, Italy. [Reprint requests]

Scott Murphy, MD, Chief Medical Officer, American Red Cross Blood Services, Penn-Jersey Region, Philadelphia, PA.

BEST membership in September 1994

Chairman: G. Sirchia, Milan, Italy

Executive Committee: C.Th. Smit Sibinga (Vice-Chairman, liaison with ISBT), Groningen, the Netherlands; B. Wenz (Vice-Chairman), Bronx, NY; G.N. Vyas, San Francisco, CA; P. Rebulla (Scientific Secretary), Milan, Italy

Full Scientific Members (and associate members): M.A. Blajchman, Hamilton, ON, Canada; W.H. Dzik, Boston, MA; J.C. Fratantoni (liaison with Food and Drug Administration), Bethesda, MD; T.J. Greenwalt, Cincinnati, OH; W.H. Heaton, San Francisco, CA; C. Högman, Uppsala, Sweden; M. Masse, Besançon, France; H.T. Meryman, Rockville, MD; L. Messeter, Lund, Sweden (A. Chester); G. Moroff, Rockville, MD (R.R. Stromberg); N. Müller, Essen, Germany; S. Murphy, Philadelphia, PA (S. Holme, Norfolk, VA); G. Myllylä, Helsinki, Finland; R. Pietersz, Amsterdam, the Netherlands; P. Rebulla, Milan, Italy (F. Bertolini); S. Sekiguchi, Sapporo, Japan (T. Takahashi); S. Slichter, Seattle, WA; C.Th. Smit Sibinga, Groningen, the Netherlands; E.L. Snyder, New Haven, CT; G.N. Vyas, San Francisco, CA (B.D. Rawal); B. Wenz, Bronx, NY (D. Ciavarella, Valhalla, NY)

Advisory Member: H.A. Perkins, San Francisco, CA

Full Manufacturing Members: K. Ohno, Asahi, Tokyo, Japan; D.H. Buchholz, Baxter, Round Lake, IL; W.H. Walker, Biotrans/NPBI, Dreieich, Germany (M. Ras, Amstelveen, the Netherlands); R. Schuyler, COBE BCT Inc., Lakewood, CO (L. Dumont); J.L. Peterson, Haemonetics, Nyon, Switzerland; C.F. Treppa, Miles Inc., Covina, CA (R. Carmen); S. Ishida, Nissin, Osaka, Japan (E. Dehaes, Zaventem, Belgium); S.T. Wortham, Pall, Glen Cove, NY (H. Brandwein, Port Washington, NY); A. Takahashi, Terumo, Kanagawa, Japan.