An *In Vitro* Method to Quantitate Gaseous Microemboli Production of Bubble Oxygenators

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Abstract

An *in vitro* method to collect and quantify volumes of gaseous microemboli generated by a bubble oxygenator has been developed. Gaseous microemboli generated by a bubble oxygenator are captured and collected in a vortex separation test cell. The cells are pressurized and the resultant pressure changes are converted to volume gas readings using a standard calibration curve.

A doppler type ultrasonic detector and this new technique were compared in order to determine correlation between the two techniques. Results of this study indicate that the ultrasonic detector "counts" do not quantitatively relate to volume of air captured in the test cells. The placement of the ultrasonic detector within the circuit can alter its readings, e.g., proximity to turbulent areas caused by connectors or positive/negative pressure excursions due to the blood pump. Significant differences were noted when fresh, bovine blood with physiologically balanced gases were substituted for lactated Ringer's solution and 100% oxygen due to differences in bubble stability and saturation levels.

Based on these findings, the use of vortex collection cells can provide a more accurate profile of gaseous microemboli production by various cardiopulmonary devices. A circuit and procedure are described for this purpose.

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Introduction

The production of gaseous microemboli by cardiopulmonary devices[1,2] and possible deleterious effects[3,4] are well known. Several devices and procedures have been introduced to measure gaseous microemboli generated by an extracorporeal circuit[5,6]; however, a serious drawback has been the inability of the user to calibrate the instrument or procedure. Protocols describing techniques to produce calibration bubbles require equipment not generally available to most laboratories and rely on either doppler-type detectors or particle counters to verify size[7]. Latex, glass or silicon beads are often used to calibrate these devices but accurate simulation of gaseous microemboli is not possible because the former are rigid, non-pliant and cannot combine to form larger bubbles. The procedure described in this paper incorporates a standard, reproducible calibration step using air which permits quantitative volume measurements of gaseous microemboli.

Gaseous microemboli volumes are measured by taking advantage of Boyle's Law which states that as a fixed volume (V) of an ideal gas is subjected to an increase or decrease in pressure (P), it follows the equation:

$$PV = k \text{ (where k is constant dependent on temperature)} \quad [1]$$

As force is exerted on a bubble by a volume of incompressible liquid in a closed system, the pressure of the gas inside the bubble increases as the volume of gas decreases. Using this principle, pressure changes within rigid chambers can be correlated to gas volume changes.

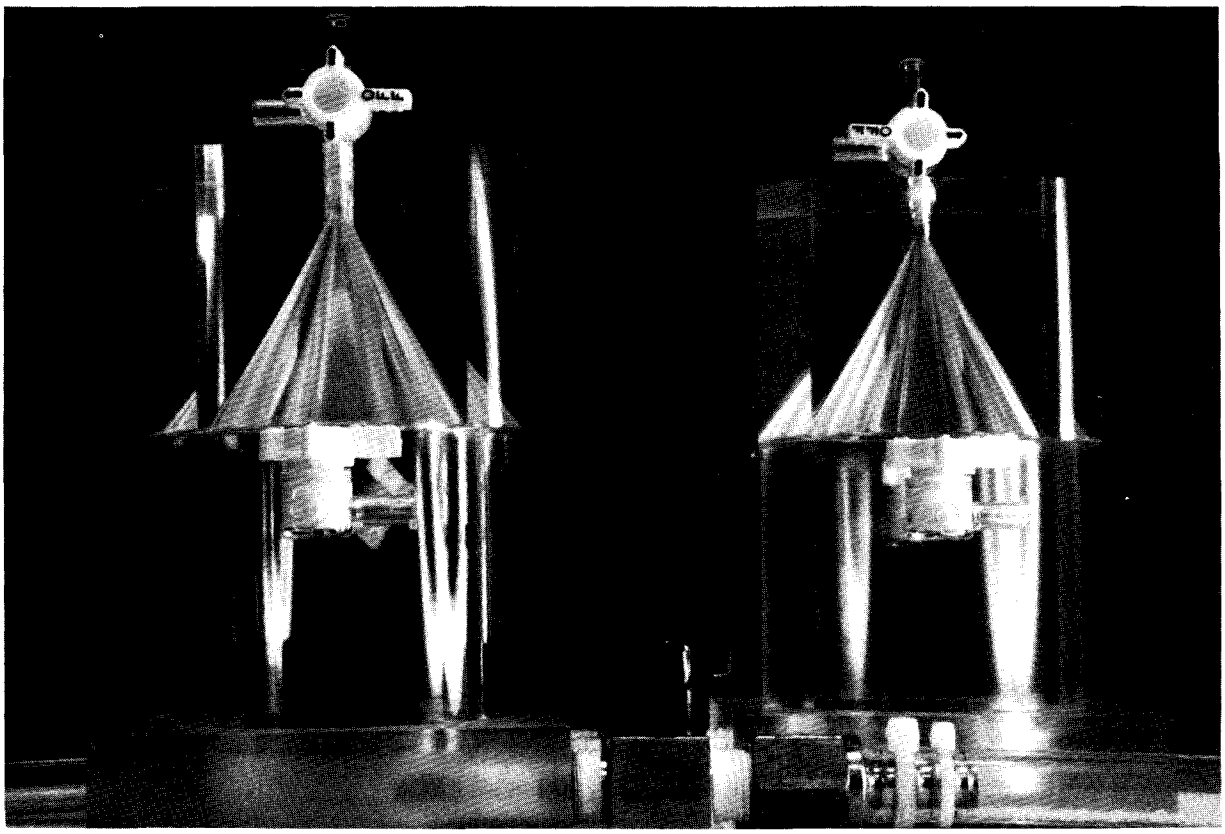

FIGURE 1: Vortex Separation Cell. Composed of clear polycarbonate blocks and joined in-tandem with stainless steel plug valves.

The test cell (Figure 1) was designed to maximize the vortex flow. This acceleration traps the bubbles entering the cell. Data accumulated during development of the cells indicate that these cells can effectively capture and retain bubbles. At specific times, the cell is isolated from the circuit and pressure readings are taken by pressurizing the cell with a known volume of fluid. A pressure transducer attached to the cell is used to measure these pressure changes which are recorded on a strip chart recorder.

The majority of previous studies conducted to evaluate gaseous microemboli production by bubble oxygenators have utilized doppler type ultrasonic detectors. The theory and mechanisms of these devices have been extensively documented[8]. Saline or lactated Ringer's solution and occasionally water appear to be the test media of choice. In nearly all cases, the ventilating gas has been 100% oxygen. While preliminary studies were conducted with lactated Ringer's solution and 100% oxygen, the bulk of the data presented

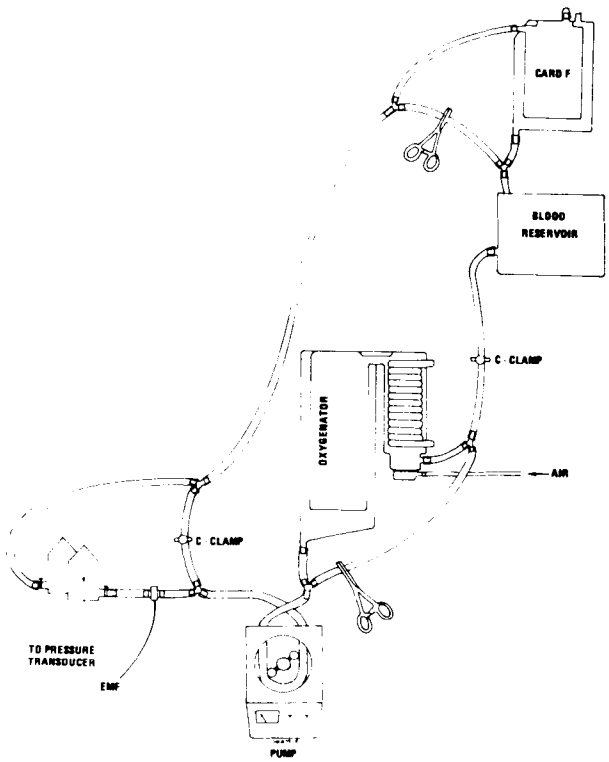

FIGURE 2: Gaseous Microemboli Test Circuit.

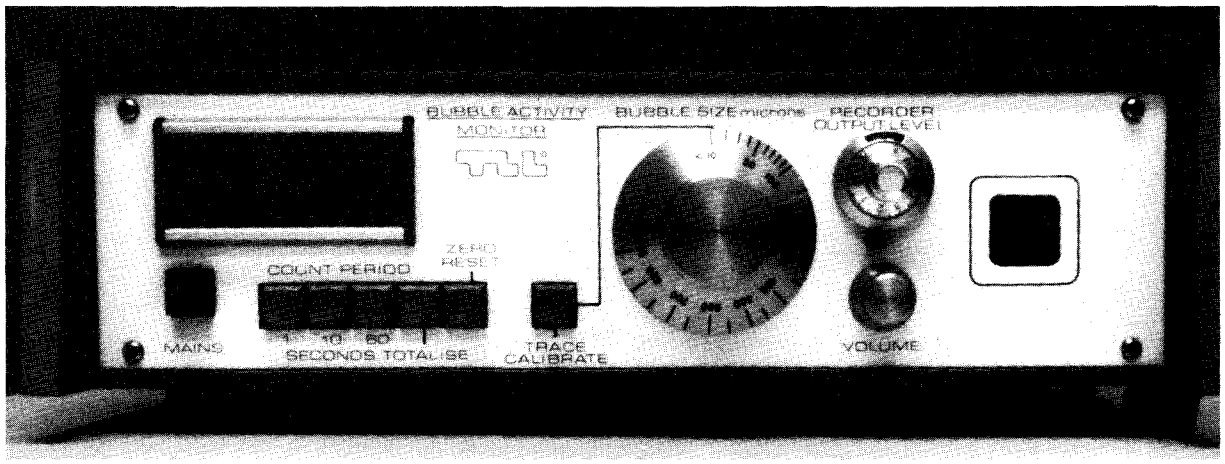

FIGURE 3: Technique Laboratories' TM-8 Bubble Activity Monitor. Settings on "Bubble Size Microns" dial appears to represent instrument sensitivity, not actual bubble size.

here was obtained using fresh, heparinized bovine blood and a modified room air gas mixture composed of 21% oxygen, 74% nitrogen, and 5% carbon dioxide. This gas mixture was selected so that "normal" arterial PO_2 values could be maintained. Significant differences in the bubble activity and volume of air collected were found when the test media was altered.

Methods

Figure 2 illustrates the test circuit used in this study. Gravity flow of blood into the oxygenator was regulated by maintaining a constant arterial reservoir level and measured by monitoring the RPM of a calibrated blood pump. The TM-8 Microbubble Activity Monitor[a] (Figure 3) was used to measure bubble activity at various locations on the test circuit. Blood flow into the vortex separation cells was monitored using an in-line electromagnetic flowmeter[b]. Pressure transducers[c] were attached on each of the vortex separation test cells and pressure change deflection curves were recorded on a multi-channel strip chart recorder[d]. Blood bypassed from the test cell was de-bubbled by the cardiotomy reservoir[e] and returned to the blood reservoir. This de-bubbling was necessary to produce the zero counts as found in venous return lines of ex-vivo circuits.

The test circuit was primed with either lactated Ringer's solution or blood adjusted to a 35% hct. The vortex separation test cells were isolated from the circuit and residual bubbles were evacuated. Additional fluid was added to completely fill the cell. Full scale and baseline deflections ("zero" air volume) were obtained by carefully injecting and withdrawing 50 μl of fluid into the cell. After attaining a consistent "zero" the calibration curve was generated.

The calibration curve was obtained by carefully introducing measured volumes of air into the primed, bubble free test cell. Using a gas-tight Hamilton syringe, 1 μl aliquots of air were introduced into the test cell. After each injection, 50 μl of fluid were introduced and removed to compress and decompress the air. The subsequent change in the cell's pressure was recorded on the strip chart recorder. After 10 μl of air had been injected in this manner, 90 μl of air were injected in 10 μl aliquots followed by 900 μl of air in 100 μl aliquots. This produced a calibrated standard curve ranging from 1 μl to 1000 μl (Figure 4). The length of the deflection could be correlated to the volume of air trapped in the cell. The cell was then re-primed to remove the air, re-zeroed and isolated from the circuit in preparation for the test.

The oxygenators were tested for one hour each at gas to blood flow ratio (V:Q) = 0.5:1, 1:1 and 2:1, where blood flow (Q) = 4 liters/minute. Temperature was maintained at 37°C ± 1°C using the

[a] Technique Laboratories, Hants, England
[b] Model 501, Carolina Medical Electronics, King, North Carolina 27021
[c] Model P50, Gould Statham Instruments, Inc., Hato Rey, Puerto Rico 00919
[d] Model 6720, Wantanabe Instruments Corp., Tokyo, Japan
[e] CARDF Cardiotomy Reservoir, Shiley, Inc., Irvine, California 92714

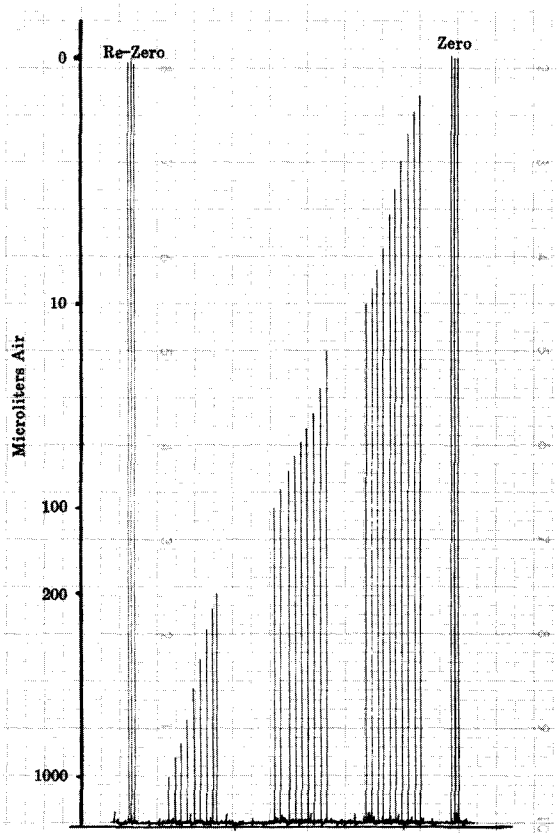

FIGURE 4: Standard Calibration Curve.

integral heat exchanger of each unit. Circuit flow rate was adjusted to the first V:Q value, and the cells were opened to the circuit. A flow of 1 liter/minute was diverted through the cells to maximize the vortex action. When pressure readings were taken, the cells were isolated from the circuit for approximately 1 minute. Pressure readings were recorded at 15 minute intervals by injecting and removing 50 μl of fluid. TM-8 readings were also recorded at each different V:Q tested.

A typical pressure tracing from one test is shown in Figure 5. These tracings were converted to air volumes using the standard calibration curve generated at the beginning of the test. To calculate the total volume of gaseous microemboli generated, the following formula was used:

$$V_T = 100A/bt \qquad [2]$$

where V_T = total volume gaseous microemboli generated per minute
A = μl of air collected in cell
b = percent of blood bypassed through the test cell
t = time in minutes

Five different adult bubble oxygenators were evaluated and compared for gaseous microemboli production and activity using the method described previously. At least three oxygenators of each type were evaluated. Two of the five oxygenators types were tested using lactated Ringer's solution/100% oxygen and fresh bovine blood/modified room air gas mixture.

Results

The TM-8 bubble activity in the lower bubble size range was higher when lactated Ringer's solution was used (Table 1). However, unlike bovine blood, activity dropped sharply to less than 30 per second in the 300 μm region. When bovine blood was used, significant bubble activity was monitored over the entire size range. The effect of V:Q variations was also more apparent with bovine blood, especially in the larger bubble size region. The stability of bubbles was further affected by positive or negative pressure (Table 2). Earlier measurements made during evaluation of infant bubble oxygenators showed that the negative pressure before a pump head pulled gas out of so-

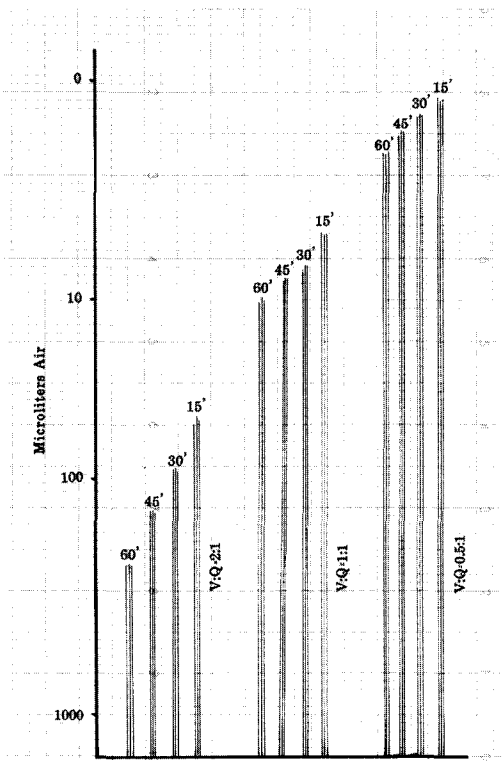

FIGURE 5: Pressure Tracings.

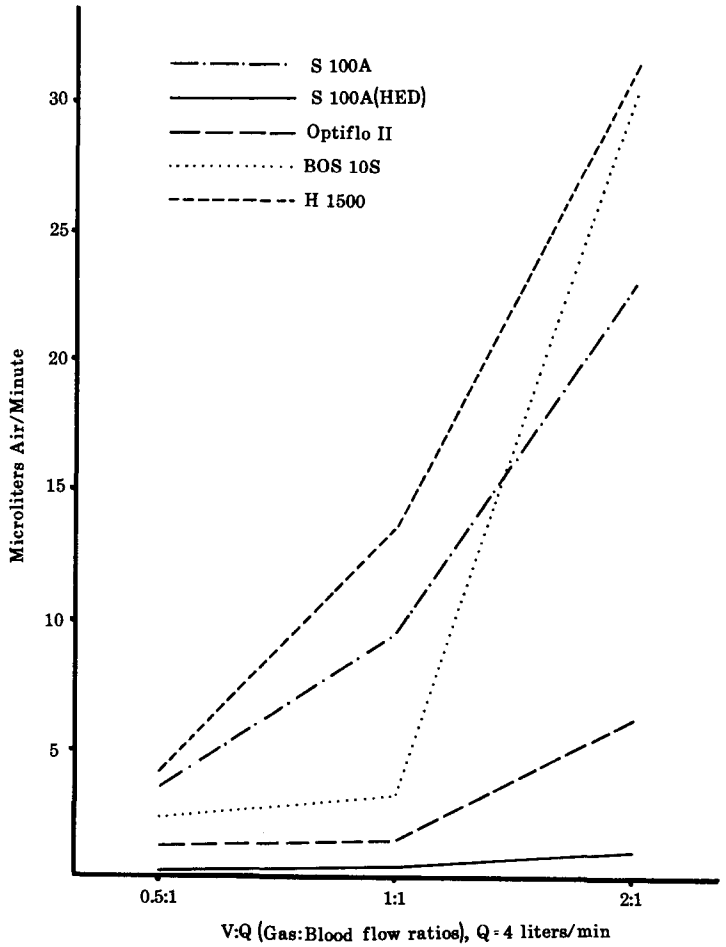

FIGURE 6: Gaseous Microemboli Production by Five Adult Bubble Oxygenators.

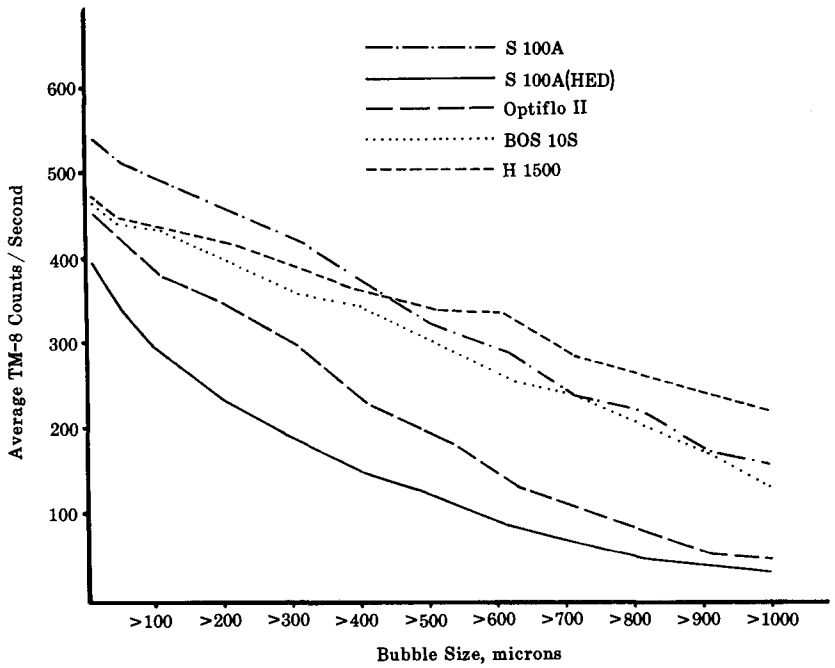

FIGURE 7: TM-8 Bubble Activity of Five Adult Bubble Oxygenators.

TABLE 1A

Comparison of Average TM-8 Bubble Activity/Second Using Lactated Ringer's and Bovine Blood

Bubble Size Microns	V:Q = 0.5:1		V:Q = 1:1		V:Q = 2:1	
	Ringers	Blood	Ringers	Blood	Ringers	Blood
10	564	525	558	513	707	506
50	206	491	164	495	246	493
100	111	452	86	474	90	489
200	47	371	23	439	23	477
300	24	319	8	403	4	457
400	14	262	3	356	0	441
500	6	217	1	318	0	427
600	3	179	0	296	0	406
700	1	149	0	246	0	378
800	0	126	0	211	0	345
900	0	103	0	184	0	326
1000	0	90	0	163	0	308

V:Q (Gas to Blood Flow Ratio) Q = 4 liters/minute
Shiley S-100A Oxygenators Tested = 5

TABLE 1B

Comparison of Average TM-8 Bubble Activity/Second Using Lactated Ringer's and Bovine Blood

Bubble Size Microns	V:Q = 0.5:1		V:Q = 1:1		V:Q = 2:1	
	Ringers	Blood	Ringers	Blood	Ringers	Blood
10	559	389	546	370	553	383
50	250	339	308	323	341	358
100	111	289	179	280	186	344
200	29	225	77	224	72	325
300	6	176	31	182	25	288
400	1	131	18	149	10	258
500	0	114	9	126	5	239
600	0	90	5	98	1	207
700	0	69	2	75	0	177
800	0	51	1	56	0	144
900	0	47	0	46	0	127
1000	0	41	0	37	0	108

V:Q (Gas to Blood Flow Ratio) Q = 4 liters/minute
Shiley S-100A (HED) Oxygenators Tested = 5

TABLE 2

Gaseous Microemboli Activity/Second
Based on TM-8 Sensor Location

TM-8 Sensor Placement	Bubble Size >50 μm	>100 μm
Oxygenator Arterial Reservoir Outlet	118 ± 23	27 ± 5
Before Pump Head	225 ± 14	43 ± 7
After Pump Head	101 ± 13	21 ± 6
Vortex Cell Inlet	49 ± 8	8 ± 2
Vortex Cell Outlet	0	0

Test Solution, Lactated Ringer's Solution and 100% Oxygen
V:Q = 1:1 where Q = 2 liters/minute
50% bypass into vortex test cells
N (Infant-type bubble oxygenators tested) = 5

lution so that an increase in bubble activity resulted. A positive pressure caused the gas to return to the solution, resulting in a lower bubble activity after the pump head.

Fresh, bovine blood with physiologically balanced gases produced bubble activity profiles which could be used to characterize the different oxygenator types. Some oxygenators had lower bubble activity in the higher bubble size range compared to other units which was not apparent when non-blood solutions were used (Table 3).

The TM-8 Bubble Activity Monitor indicated that increased numbers of larger bubbles were stabilized when blood was used. Attempts to convert the TM-8 bubble activity levels to bubble vol-

TABLE 3
Average Gaseous Microemboli Activity/Second, per Bubble Size
Based on TM-8 Readings Using Bovine Blood

Oxygenator	N	V:Q = 0.5:1		V:Q = 1:1		V:Q = 2:1	
		>10 μm	>1000 μm	>10 μm	>1000 μm	>10 μm	>1000 μm
S-100A	5	525	90	513	163	506	308
S-100A (HED)	5	389	41	370	37	383	108
Optiflo II	4	419	4	431	59	461	74
BOS 10S	4	421	62	442	153	488	344
H-1500	3	413	33	439	213	440	301

Q = 4 liters/minute
N = Units Tested

umes could not be correlated to the actual air volumes collected by the vortex cells (Table 4). This was probably due to coincident counting of several small bubbles as a single large bubble and/or counting several bubbles several times by the TM-8.

Discussion

A major problem encountered during the evaluation of cardiopulmonary devices, specifically bubble oxygenators, has been the precise quantification of bubble number and size which are introduced into the arterial line. Ultrasonic counters/detectors cannot be easily calibrated by the user and are subject to coincident counting, the inability to distinguish one large bubble from a mass of small bubbles and possible indiscriminate counting of bubbles and particles. The combination of these factors probably contributed to the lack of correlation between bubble counts and actual bubble volumes found in this study. These detectors generally rely on a clean, non-blood solution to perform efficiently. While this eliminates the particles vs. bubbles problem, this study indicates that non-blood primes cannot support bubbles in the same manner as blood due to differences in viscosity, surface tension and gas saturation levels. Many of the bubbles are never detected because they do not form in a stable manner and redissolve or rise and break at the surface of the arterial reservoir when non-blood solutions are used.

Comparative analysis between bubble detection methods is difficult since insufficient attention has been given by investigators to the various limitations of each method. It is much easier to "count" and accept as fact the data generated by ultrasonic type detectors without making a critical analysis of potential interactions. This new procedure can approach quantification in a more precise manner than other methods. Incorporation of timed gas emboli volume collection with properly analyzed ultrasonic counters can provide a more realistic profile of bubble activity.

TABLE 4
Average Gaseous Microemboli Production,
(μl/minute) Based on Vortex Test
Cell Readings Using Bovine Blood

Oxygenator	N	V:Q = 0.5:1	V:Q = 1:1	V:Q = 2:1
S-100A	5	2.7	10.4	24.5
S-100A (HED)	5	0.0	0.1	0.9
Optiflo II	4	1.2	1.3	6.7
BOS 10S	4	1.8	2.9	33.8
H-1500	3	3.3	14.4	34.1

Q = 4 liters/minute
N = Units Tested

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