

## Surfactant protein A and stable microbubble formation in tracheal aspirates

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### Abstract

This study aimed to determine the role of surfactant protein A (SP-A) in the formation of stable microbubble in tracheal aspirates. Our results showed that as the concentration of anti SP-A antibodies added to tracheal aspirate specimens increased, the number of stable microbubble formed in the specimen decreased. The correlation between stable microbubble counts and the SP-A levels in the tracheal aspirates was good,  $r = 0.85$ ,  $p < 0.05$ . This study suggests that SP-A plays an important role in stable microbubble formation. Measurement of small stable microbubbles is thus a useful bedside test for predicting the SP-A activity in the tracheal aspirates and in indirect measurement of lung maturity.

**Key words:** Stable microbubble, SP-A, tracheal aspirates

### INTRODUCTION

Pulmonary surfactant stabilizes the alveoli by lowering the surface tension during the expiratory phase of breathing, thus preventing alveolar collapse.<sup>1</sup> The optimal activity of lung surfactant depends on the presence of specific proteins.<sup>2</sup> Surfactant associated protein A (SP-A) remains the major component of surfactant proteins.<sup>3</sup> It reacts synergistically with phospholipid mixture<sup>4,5</sup> and in the regulation of surfactant phospholipid in human alveoli.<sup>6,7</sup> This study was carried out in order to assess the important of SP-A in stable microbubble formation and whether the measurements of small stable microbubbles reflect the concentration of SP-A in tracheal aspirates and, therefore, lung maturity.

### METHODS AND MATERIALS

#### *The stable microbubble (SM) test*

The SM test was performed on one of the tracheal aspirate specimens obtained from neonates in the intensive care unit, UKM according to Pattle *et al.*<sup>8</sup> An aliquot of tracheal aspirate was aspirated up to 5 cm of the stem of a Pasteur pipette which had a lumen of 1 mm in diameter and a stem of 11.5 cm long (Pyrex, Coming Laboratory Science Co., New York, USA) using a 2 ml rubber cap. The tip of the pipette was then placed vertically on a 50 mm x 35 mm microscope repeatedly for 20 times in quick succession. The microscope slide was then inverted so that a hanging drop of the tracheal aspirate was formed. After 4 minute, the slide

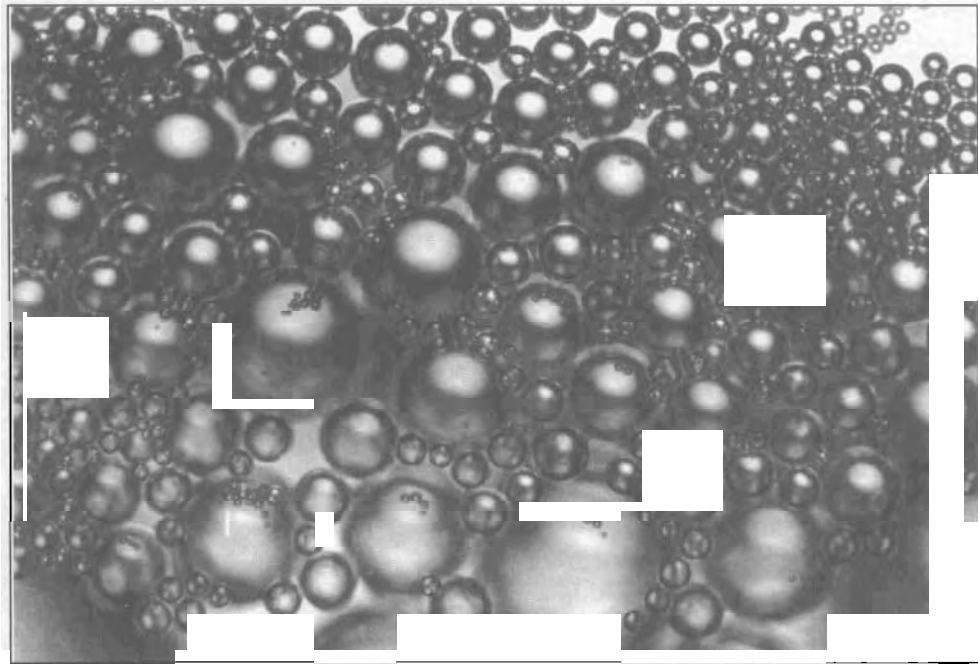
was examined under the 10x objective of a microscope using 1 mm<sup>2</sup> field and a 15µm scale. Counts were made in five regions: the four quadrants of and the centre of the bubble field. All stable microbubbles of less than 15µm in diameter were counted. The mean count of microbubble per mm<sup>2</sup> of the five regions were calculated.

The SM count was repeated on the same tracheal aspirate specimen each time after dilution with the in-house polyclonal rabbit anti SP-A<sup>9</sup> (dilution 1:500) in phosphate buffer saline in the ratio of 1:1 (v/v).

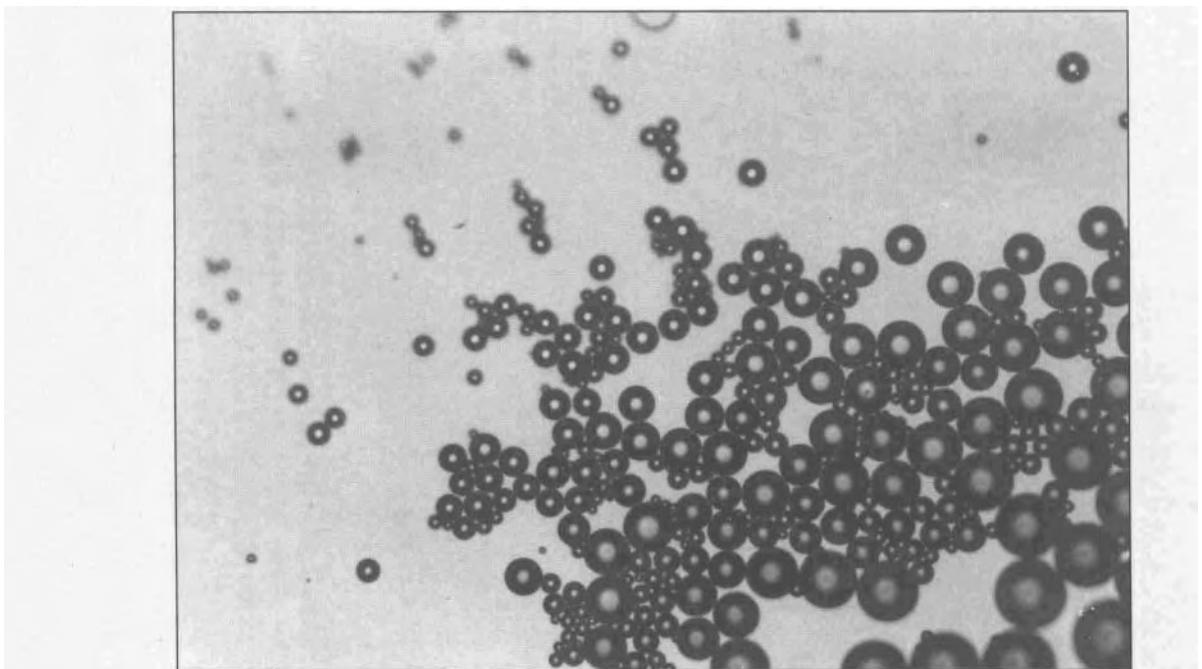
Bovine serum albumin with the same protein concentration as the tracheal aspirate specimen measured by the uv method<sup>10</sup> was used as control. The same SM test was performed on this control before and after each dilution with the polyclonal rabbit anti SP-A as described above.

#### *Comparison between the results of in-house ELISA for SP-A with SM test*

104 tracheal aspirate specimens were obtained from neonates with and without respiratory distress syndrome in the intensive care unit, UKM. The concentration of SP-A in these tracheal aspirates was measured with the in-house ELISA" by one of the authors without knowledge of the SM count results or clinical condition of the patients. The results obtained were compared with the SM test performed on each specimen as described above (Figures 1 & 2) by one of the authors (NYB).



**FIG. 1:** Photograph showing specimen with more than 200 stable microbubbles under a x 10 objective microscopic field.



**FIG. 2:** Photograph showing specimen with about 50 - 60 stable microbubbles under a x 10 objective microscopic field.

**Statistical analysis**

The student t test and simple regression analysis were performed for comparing the stable microbubble test with the in-house ELISA method using the Statgraphic program (STSC, Inc, USA).

**RESULTS**

The results obtained of the SM test on the tracheal aspirate specimens and bovine serum albumin with and without addition of polyclonal anti SP-A are shown in Table 1 and Table 2 respectively.

The neat tracheal aspirate specimen contained more than 200 stable microbubbles and as progressive dilutions was made to the specimen the number of stable microbubbles reduced accordingly. Furthermore, the addition of

increasing concentrations of polyclonal rabbit anti SP-A to the above specimen abolished stable microbubbles formed as well.

The control specimen containing bovine serum albumin at the same initial protein concentration as the tracheal aspirate specimen formed very few or no stable microbubble compared to the tracheal aspirate specimen. The subsequent addition of anti SP-A did not affect the number of stable microbubble formed.

The correlation between the SM test and in-house ELISA was shown in Figure 3. There was good correlation between the two methods with a correlation coefficient of 0.85 and R-squared of 73.05%,  $p < 0.05$ .

**DISCUSSION**

Our results showed that as the concentration of anti SP-A in the tracheal aspirate specimen

**TABLE 1: Number of stable microbubble formed by mixing progressive dilutions of one of the tracheal aspirate samples with progressive dilutions of anti SP-A**

Progressive dilutions of anti SP-A	Progressive dilutions of tracheal aspirates						
	1:2	1:4	1:8	1:16	1:32	1:64	1:128
None	240.0	200.0	164.2	124.0	68.0	32.0	14.0
1:10	1.6	0.8	0.4	0.0	0.0	0.0	0.0
1:20	3.2	1.4	0.8	0.4	0.0	0.0	0.0
1:40	6.4	2.8	1.2	0.8	0.4	0.0	0.0
1:80	12.6	5.6	3.2	1.6	0.8	0.0	0.0
1:160	25.0	10.4	7.4	2.4	1.2	0.4	0.0
1:320	48.0	20.0	14.0	4.8	2.8	0.8	0.4
1:640	96.0	40.0	30.0	9.2	4.6	1.6	0.8
1:1280	148.0	86.0	62.0	18.8	6.4	3.6	1.2
1:2560	180.0	150.0	88.8	32.0	13.2	7.8	2.8
1:5120	200.0	172.2	120.2	62.6	32.4	16.0	6.4
1:10240	238.0	210.0	160.4	110.0	64.0	30.2	12.0

NB: The number of stable microbubble is a mean of counts from 5 fields (x100 magnification)

**TABLE 2: Number of stable microbubble formed by mixing progressive dilutions of a bovine serum albumin (BSA) sample with progressive dilutions of anti SP-A**

Progressive dilutions of anti SP-A	Progressive dilutions of bovine serum albumin (BSA)						
	1:2	1:4	1:8	1:16	1:32	1:64	1:128
None	2.4	0.0	0.0	0.0	0.0	0.0	0.0
1:10	2.4	0.0	0.0	0.0	0.0	0.0	0.0
1:20	2.4	0.0	0.0	0.0	0.0	0.0	0.0

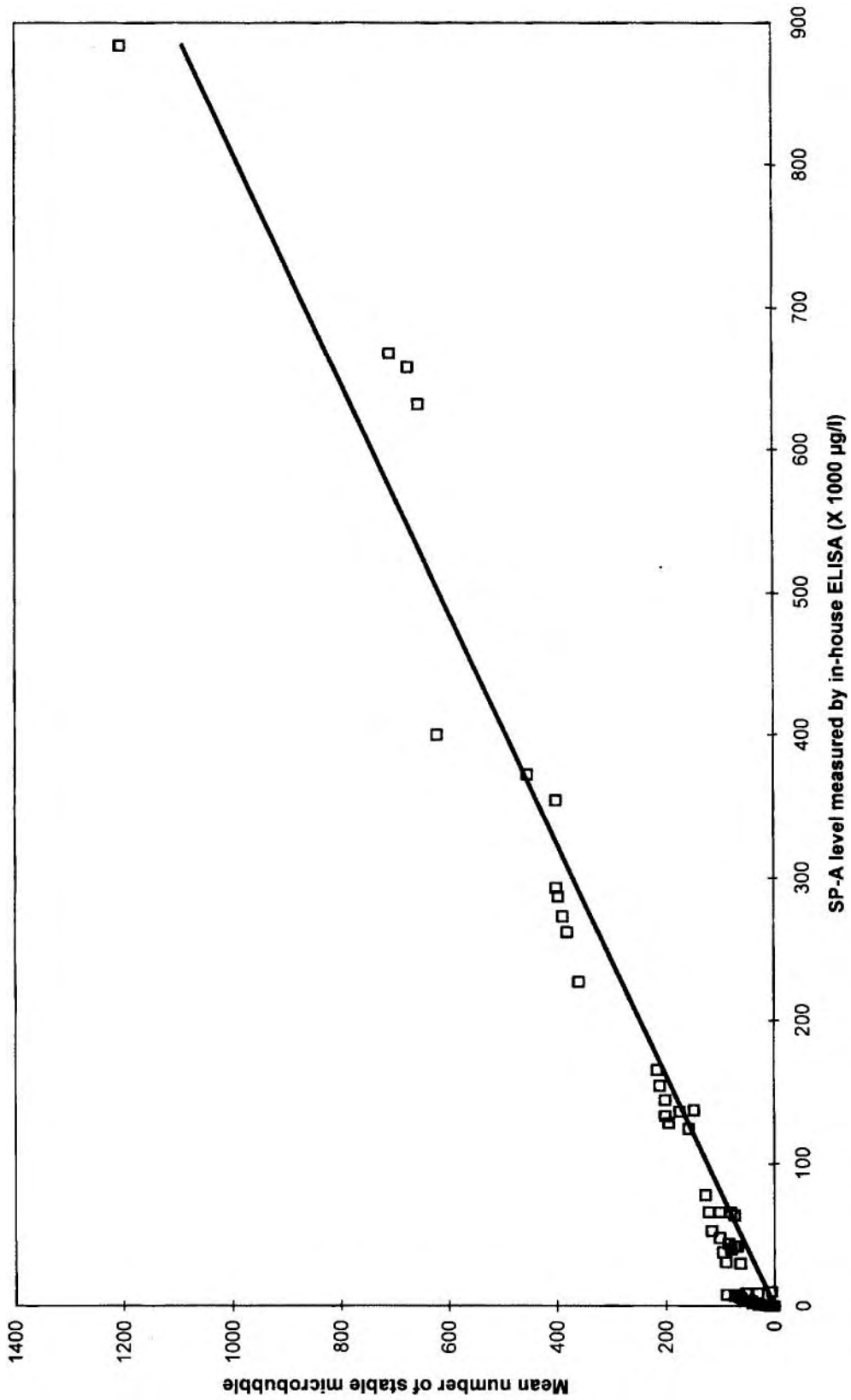


FIG. 3: Comparison studies on in-house ELISA and stable microbubble test.

increased, the number of stable microbubble formed decreased. In the control specimen containing bovine serum albumin without SP-A, few or no stable microbubbles were formed with and without the addition of anti SP-A. This shows that SP-A is required for stable microbubble formation.

The SM test on tracheal aspirates shows good correlation with SP-A level in aspirates measured by the in-house ELISA method. Thus measurement of small stable microbubbles predicts the SP-A activity of the tracheal aspirates. This has important clinical application as the SM test is a bedside test and the result can be available immediately, whereas ELISA takes several days before the result is known. Our study suggests that the SM test on tracheal aspirates is a useful bed-side test to determine lung maturity.

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