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Efficacy of Dye-Stained Enteral Formula in Detecting Pulmonary Aspiration

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Abstract

Study objective—To determine the extent to which a mixture of human gastric juice and enteral formula stained with two concentrations of FD&C Blue No. 1 food dye (0.8 and 1.5 mL/L) is visible in suctioned tracheobronchial secretions following three forced small-volume pulmonary aspirations over a 6-h period in an animal model.

Design—Experimental 2 × 3 repeated measures.

Setting—Animal laboratory and an acute care hospital.

Participants—Ninety New Zealand white rabbits weighing approximately 3 kg each, and 90 acutely ill adults who furnished gastric juice.

Interventions—A mixture of human gastric juice and enteral formula stained with 0.8 or 1.5 mL of dye per liter was instilled intratracheally over a 30-min period into anesthetized intubated animals at baseline, 2 h, and 4 h. A total of 0.4 mL/kg of the mixture was instilled at each session. Ninety minutes after each instillation, suctioned secretions were examined for visible dye and blood.

Measurements and results—Dye was visible in 46.3% of the secretions (125 of 270). The concentration of dye had no significant effect on dye visibility. Blood that was present in 114 of 270 of the secretions (42.2%) interfered with dye visibility in all but two secretions. For reasons unknown, even in the absence of blood, dye visibility decreased from 90.2% (55 of 61 secretions) after the first aspiration event to only 61% (25 of 41 secretions) after the third aspiration event.

Conclusions—Findings from this animal model study do not support the use of the dye method to detect repeated small-volume aspirations. For clinicians who choose to use the dye method in selected situations, it appears that a dye concentration of 0.8 mL/L may be as effective in detecting aspiration as a 1.5 mL/L concentration.

Keywords

aspiration/diagnosis; enteral nutrition; food coloring agents/diagnostic use; pneumonia; rabbits

A method often used to detect aspiration in tube-fed patients consists of adding FD&C Blue No. 1 dye to the feeding solution and observing for its appearance in secretions suctioned from the airway.[1–4] However, there is little research-based evidence defining the efficacy of this

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approach. To the contrary, several small studies[5–8] have indicated that it has low sensitivity. In addition, there is little agreement as to how the method should be implemented.[9]

The amount of dye that must be added to enteral formula in order to detect aspiration is unknown. In clinical practice, it is common to add dye until the visual intensity is perceived to "look right." The reported amounts to achieve this nebulous color intensity range from as little as a few drops per 240 mL of formula up to 10 mL per bag of formula (total volume unspecified).[10] Another factor to consider is the color of the gastric juice with which the dye-stained formula becomes mixed as it is administered into the stomach. For example, while gastric juice may be clear and colorless, it is often green (from refluxed bile) or brown (from digested blood).[11] Therefore, it is conceivable that mixing a dye-stained enteral formula with heavily pigmented gastric juice could preclude the visibility of dye, negating its utility in detecting the aspiration of gastric contents into the lungs.

On the basis of animal experiments, the US Food and Drug Administration estimated that a maximum acceptable daily intake of FD&C Blue No. 1 is approximately 12.0 mg/kg body weight per day (or < 1 g/d in the average adult).[12] Whether or not this level of dye intake is acceptable for acutely ill humans is unknown. Anecdotal reports[13–15] have indicated that dye-stained enteral formula can result in a generalized discoloration of body fluids and tissues following the absorption of the dye from the GI tract. In addition, anecdotal reports[16,17] have indicated that conditions associated with increased gut permeability (such as sepsis) increase the potential for the systemic absorption of dye. Because FD&C Blue No. 1 dye has been shown to be a potent inhibitor of mitochondrial respiration *in vitro*, its use may be an unsafe practice in patients with illnesses associated with enhanced gut permeability.[17] Other dyes have a potential for harm when used as a marker for aspiration in tube-fed patients. For example, methylene blue has been associated with the development of Heinz body hemolytic anemia in infants[18] and inhibits guanylate cyclase.[19]

This study was designed to determine the extent to which a mixture of human gastric juice and enteral formula stained with two concentrations of FD&C Blue No. 1 (0.8 and 1.5 mL/L) is visible in suctioned tracheobronchial secretions following three forced small-volume pulmonary aspirations over a 6-h period in an animal model. The following research questions were addressed:

1. Does the dye concentration in an enteral formula (*ie*, 0.8 and 1.5 mL/L) have an effect on the visibility of the dye in suctioned tracheobronchial secretions?
2. Does dye visibility in suctioned tracheobronchial secretions change across 2-h, 4-h, and 6-h time points (with a forced aspiration ending 90 min before each time point), and do these changes vary by dye concentration?
3. Does the volume of suctioned tracheobronchial secretions differ at the 2-h, 4-h, and 6-h time points?
4. Is there a relationship between dye visibility in the suctioned tracheobronchial secretions and their volume?
5. Does blood in suctioned tracheobronchial secretions have an effect on dye visibility in the secretions?

Materials and Methods

Ninety New Zealand white rabbits weighing approximately 3 kg each were the primary subjects. Ninety acutely ill adults furnished gastric juice for use in the project. The study was approved by the Saint Louis University Human Subjects Committee prior to obtaining informed consent from the acutely ill adults who furnished the gastric juice used in the project.

The animal experiments were conducted according to the rules and regulations of the Saint Louis University Animal Care Committee.

On the day preceding each experiment, human gastric juice was collected from an acutely ill hospitalized adult who had been fasting for at least 4 h and had received no medications by tube or mouth within the preceding hour. None of the subjects had received dye-stained formula. Gastric juice from the 90 human subjects ranged in color from light tan or green to dark brown or green, and the pH ranged from 1.22 to 7.33 (mean ± SE, 3.97 ± 0.20). No significant difference in pH was found between gastric samples used for the low-concentration dye group and the high-concentration dye group (3.69 ± 0.30 vs 4.26 ± 0.26, respectively; $p = 0.153$). The gastric juice was refrigerated at 4°C until needed on the following day for mixing with dye-stained enteral formula to simulate conditions present in humans who are aspirating gastric contents during tube feedings.

On the morning of each experiment, the gastric juice was mixed half and half with one of eight enteral formulas that was stained with one of two dye concentrations (*ie*, 0.8 or 1.5 mL/L). A dye concentration of 1.5 mL/L formula was selected because a small clinical study[20] had indicated that this concentration of dye does not interfere with guaiac testing of fecal contents in tube-fed patients. A dye concentration of 0.8 mL/L formula was selected because it is approximately half of 1.5 mL/L and has been recommended by Davis et al[21] as a reasonable concentration of dye with which to detect aspiration without causing adverse reactions. Figure 1 depicts the color intensity of these two concentrations of the FD&C Blue No. 1. The dye used in the project consisted of 2.5% FD&C Blue No. 1 (Novartis Nutrition; Minneapolis, MN). Blue dye was selected because it is typically used in clinical settings as the dye of choice with which to detect aspiration in tube-fed patients. A 2.5% solution of FD&C Blue No. 1 dye contains 100 mg tint per 4 mL.

The eight enteral formulas (Glucerna, Jevity, Osmolite, Pediasure, Pulmocare, and Twocal HN; Abbott Laboratories; Abbott Park, IL; and Isocal and TraumaCal; Mead Johnson; Evansville, IN) were approximately evenly distributed among the two dye concentrations. A variety of enteral formulas were selected to ascertain whether dye visibility findings are formula-specific. In addition, in another segment of the study not reported herein, we measured the glucose concentration in suctioned tracheal secretions to determine the efficacy of the glucose method in detecting aspirations following the pulmonary instillation of a variety of enteral formulas with low, moderate, and high glucose levels.

The animals were anesthetized, intubated with a 3.5-mm uncuffed endotracheal tube (ETT), and mechanically ventilated (VIP BIRD Infant Pediatric Ventilator with an attached warm air respiratory humidifier; Fisher & Paykel Healthcare; Auckland, New Zealand). The animals were anesthetized with ketamine and were paralyzed with pancuronium bromide. All of the animals were in a supine position on a horizontal plane throughout the experiments. Continuous monitoring for hemodynamic and acid-base status was performed. A tidal volume of 50 mL with a positive end-expiratory pressure of 2 cm H_2O was used in all animals with the frequency altered to control acid-base balance. Forty-five animals received gastric juice mixed with formula containing 0.8 mL dye per liter, while the remaining 45 animals received gastric juice mixed with formula containing 1.5 mL dye per liter. A volumetric infusion pump was used to instill three separate boluses of the mixture intratracheally via a small catheter introduced through the ETT into the mainstem bronchus approximately 1 cm distal to the larynx and about 5 mm distal to the tip of the ETT. The position of the catheter in the ETT was not ensured by radiography; however, because the procedure for each animal was the same, the position of the catheter in relation to the ETT was constant across animals. At the beginning of the experiment, 0.4 mL/kg mixture was infused over a 30-min period. The infusion then was stopped, and 90 min were allowed to elapse before endotracheal suctioning was performed

with a 6.5F catheter attached to a 20-mL pediatric mucus trap. A 90-min time period was selected to allow the dilution of the infused mixture by local respiratory secretions. (Normal saline solution was not instilled into the trachea during the suctioning procedure to avoid the dilution of the secretions.) The suctioned material was visually inspected against a white background for the presence of blood and dye. At hour 2, an additional 0.4 mL/kg mixture of gastric juice and dye-stained enteral formula was infused over a 30-min period. Again, the infusion was stopped, and 90 min were allowed to elapse before endotracheal suctioning was performed. At hour 4, this process was repeated. Thus, by the end of the 6-h experiment, each animal had received intratrache-ally a total volume of fluid (half gastric juice and half dye-stained enteral formula) equivalent to 1.2 mL/kg body weight.

A total of 270 tracheobronchial secretions were obtained by suctioning each of the 90 animals three times. One hundred thirty-five secretions were from the low-concentration (0.8 mL/L) group, and 135 were from the higher concentration (1.5 mL/L) group. The same registered nurse research assistant visually inspected all of the suctioned tracheobronchial secretions for blood and dye, and recorded the findings as present or absent immediately after suctioning the animals. When the suctioned secretions were brought to a research laboratory for glucose and other analyses, the study biochemist recorded an opinion as to whether or not blood and dye were visible for one third of the secretions. A second nurse research assistant was present occasionally in the animal laboratory at the time of suctioning and examined the secretions at the same time as the first registered nurse research assistant. They independently recorded their observations without discussion. Identifying the presence of blood was not difficult since the preponderance of the bloody secretions were grossly bloody. While there were gradations in dye intensity, dye was deemed to be present when any blue tint was observed as the specimen was held against white paper. None of the observers were blinded to the use of dye and its concentration in the infused mixture. For the 31% of secretions in which two or more observers made ratings of dye visibility, agreement was 100%.

Data were analyzed by a combination of descriptive, nonparametric, and parametric statistics. Comparisons of the two dye concentration groups on the dependent variables of dye visibility and the presence or absence of blood in secretions were analyzed by the Mann-Whitney test. Changes in proportions over time were analyzed by nonparametric tests (*ie*, Cochran *Q* test and McNemar test). Changes in volume for the two dye concentrations over time were analyzed by a 2 × 3 repeated-measures analysis of variance. Relationships between dye visibility and volume were determined using a series of two sample *t* tests. An α level of 0.05 was used.

Results

We found that dye was visible in 125 of the 270 suctioned tracheobronchial secretions (46.3%). As shown in Table 1, the concentration of dye (0.8 and 1.5 mL/L) did not produce significant differences in dye visibility in the secretions at any time point. However, with both dye concentrations, dye visibility in the suctioned secretions changed significantly over time, being the least visible at the 6-h data collection point. When each of the dye concentrations was examined separately, the same pattern of change occurred; however, a significant decrease in dye visibility for each dye concentration did not occur until the 6-h period.

The gastric juice used for 2 of the 90 animals was dark brown. On being mixed with formula containing dye in a concentration of 0.8 m/L, the entire solution was colored brown (thereby precluding the visibility of the blue dye). A total of six secretions were suctioned from these two animals. Dye was not observed in five of these six secretions. Instead, the secretions had the color of the brown gastric juice. Dye also was not observed in the sixth specimen, which was grossly bloody.

The mean volume (± SD) of secretions that were suctioned from the 90 animals was 0.42 ± 0.46 mL. Changes in mean volumes of the suctioned secretions over the three data collection points are shown in Table 2. While the volume of secretions at 4 and 6 h were significantly greater than that at 2 h, the volumes at 4 and 6 h did not differ significantly. The absence of a significant concentration by time interaction suggests that the pattern over time is similar for the two dye concentration groups ($p = 0.65$). There was also no significant concentration main effect, suggesting that dye concentration does not affect volumes ($p = 0.26$).

Table 3 depicts the relationship between the visibility of dye in the suctioned secretions and the volumes of the secretions. When dye was not visible, there was a tendency for the volume to be higher; however, statistical significance was reached only at the 4-h data collection point. Mucus was not observed in any of the suctioned tracheobronchial secretions.

Blood was visible in 42.2% of the secretions (114 of 270 secretions) and was approximately evenly distributed across the low-concentration and higher concentration groups (58 of 135 secretions and 56 of 135 secretions, respectively). Table 4 shows the effect of blood in the secretions on the ability to observe dye. As can be seen, blood in the secretions greatly interfered with the ability to visualize dye. That is, dye was visible in 78.8% of the nonbloody secretions (123 of 156 secretions), as opposed to only 1.8% of the bloody secretions (2 of 114 secretions). In the latter two cases, the secretions were blood-streaked and dye was visible in portions of the secretions that were not stained with blood.

Although the higher dye concentration did not result in a greater presence of blood in the suctioned tracheobronchial secretions, we found that bloody secretions were associated with high pH in the human gastric juice that was instilled into the animals' lungs at all time intervals. For example, the mean gastric fluid pH at the first suctioning was 4.92 when bloody tracheobronchial secretions were obtained, as opposed to 3.52 when nonbloody secretions were obtained ($p = 0.001$). A similar pattern was observed at the second suctioning ($p = 0.003$) and at the third suctioning ($p = 0.000$). This may have been because the high pH gastric juice contained materials that had been refluxed from the small bowel, such as trypsin and bilirubin.

Discussion

The overall sensitivity of the low dye concentration in detecting aspirations did not differ significantly from that of the higher dye concentration (44.4% [60 of 135 secretions] vs 48.2% [65 of 135 secretions]). Thus, there appears to be little benefit from using the higher dye concentration.

Dye visibility deteriorated sharply over the 6-h study period. In the entire sample, dye visibility dropped from 62.2% at the 2-h suctioning (56 of 90 secretions) to 27.8% at the 6-h suctioning (25 of 90 secretions). Because the study's high proportion of bloody secretions caused the visibility of dye in the entire sample to be quite low, we also reported the percentage of dye visibility in secretions that did not contain visible blood. Even then, however, dye visibility diminished significantly from about 90% at 2 h to about 61% at 6 h. This occurred despite intratracheal bolus injections of dye-stained formula at 2-h intervals. Given the sharp decline in dye visibility over the 6-h study period, a question arises as to the efficacy of the dye method over a longer period of time. It is difficult to explain why the visibility of the dye diminished over time, although the volume of suctioned secretions was larger when the dye was not visible at 4 h, and volume and visibility were not significantly associated at 2 or 6 h.

The major strength of the study was the ability to precisely control the time of aspiration, the amount of fluid aspirated, the concentration of the dye that was used in the aspirated feeding solution, and the time of secretion retrieval from the animals' lungs. In contrast to studies that evaluated the dye method in humans when it was uncertain whether aspiration had actually

occurred, this study had the advantage of evaluating tracheal secretions for dye visibility following three separate forced aspiration events. Further, to more closely simulate what occurs during aspiration in tube-fed humans, we were able to mix the dye-stained formula with human gastric juice.

The extent to which the findings from this animal model study can be extrapolated to humans is unknown. Furthermore, a number of limitations were inherent to the study and deserve consideration. The percentage of bloody tracheobronchial secretions in the animals (42% [114 of 270 secretions]) was much higher than that typically observed in suctioned secretions from the airways of humans. For example, in an earlier study[22] in which tracheobronchial suctioning was performed on 84 acutely ill adults, we found a substantially lower percentage of bloody secretions (11% [9 of 84]). This difference has significance because blood greatly interfered with the ability to see dye in the suctioned secretions. Because the observers were not blinded to the dye conditions, it is conceivable that their perception of the dye was falsely heightened over what might occur in a clinical setting where an observer is unaware of when patients are aspirating dye-stained formula.

Although rabbits have goblet cells that are capable of secreting mucus, they do not do so to the extent that is found in intubated humans (who can form mucus freely following pulmonary irritation by a foreign substance). From this perspective, it would be reasonable to speculate that dye visibility in human tracheobronchial secretions might be even lower than that observed in this animal model study. The mixture of dye-stained formula and human gastric juice was instilled through the animals' ETTs, rather than around the tube, as would occur in intubated, tube-fed humans. However, it is unlikely that this factor had a significant effect since the dye-stained mixture would enter the lung by either route.

We have no evidence that all of the eight formulas used in the study behave the same way when exposed to gastric juice for 2 to 6 h. Thus, the variety of formulas may have been a confounding variable. However, the formulas were used in similar numbers according to dye concentration groups; thus, any variance was likely to be distributed equitably. Furthermore, dye visibility did not differ significantly according to formula type.

Another possible confounding variable is the length of time that gastric juice and formula were mixed prior to instillation into the animals' lungs. As indicated earlier, gastric juice was mixed with the dye-stained formula immediately prior to the experiment. Part of the mixture was used for the first instillation, and the rest was refrigerated until used for the second and third instillations. Thus, time 1 evaluated the immediate exposure of gastric juice and aspiration, as might occur in patients with poor lower esophageal sphincter tone, which allows them to continuously reflux and aspirate small amounts of feedings. Times 2 and 3 evaluated both the duration of the *ex vivo* effect of gastric juice on dye-stained formula and the effect of aspiration, as might occur in patients who have slowed GI motility that allows feedings to accumulate in the stomach for hours before being aspirated.

Conclusion

The dye method's overall sensitivity in detecting multiple forced aspirations in this animal model study was low (46.3% [125 of 270 secretions]). When only nonbloody secretions were considered, the overall sensitivity of the method was modest (78.8% [123 of 156 secretions]). The precipitous drop in dye visibility from the first aspiration event to the third aspiration event casts serious doubt on the ability of this approach to detect repeated aspirations. When these findings are coupled with anecdotal reports of potential harm from the use of the dye method,[13–18] there appears to be little justification for its use. For clinicians who choose to use the

dye method in selected situations, it appears that a dye concentration of 0.8 mL/L formula is as effective as a concentration of 1.5 mL/L.

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Abbreviation

ETT

endotracheal tube

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Figure 1.
Dye-stained formula. At left is a beaker containing the formula, but no dye. In the center is a beaker containing the formula and FD&C Blue No. 1 dye at a concentration of 0.8 mL/L. At right is a beaker containing the formula and FD&C Blue No. 1 dye at a concentration of 1.5 mL/L.

Table 1

Effect of Two Dye Concentrations in Enteral Formula on Visibility of Dye in Suctioned Tracheobronchial Secretions Over Three Time Points*

	Suctioned Secretions in Which Dye Was Visible (n = 45)		
Dye Concentration, mL/L	**2 h**	**4 h**	**6 h**
0.8	57.8 (26)	46.7 (21)	28.9 (13)†
1.5	66.7 (30)	51.1 (23)	26.7 (12)†
Average of the two Concentrations‡	62.2	48.9	27.8

*Values given as % (No. of secretions in which dye was visible).

†At 6 hours, visibility of dye was significantly lower ($p < 0.05$) than at 2 and 4 h.

‡Average visibility of dye decreased significantly ($p < 0.05$) at each successive time point.

Table 2

Change in Volumes of Suctioned Tracheobronchial Secretions Over Time by Dye Concentration*

Dye Concentration, mL/L	Suctioned Tracheobronchial Secretions, mL		
	2 h	4 h	6 h
0.8	0.20 ± 0.17	0.45 ± 0.88†	0.44 ± 0.39†
1.5	0.26 ± 0.22	0.56 ± 0.80†	0.61 ± 0.60†

*Values given as mean ± SD.

†Significantly greater ($p < 0.05$) than mean volume at 2 h.

Table 3

Relationship Between Dye Visibility and Volume of Suctioned Tracheobronchial Secretions

Condition	Suctioned Secretions, mean ± SD	p Value
2 h		
Dye not visible (n = 34)	0.25 ± 0.20	NS*
Dye visible (n = 56)	0.21 ± 0.20	
4 h		
Dye not visible (n = 46)	0.72 ± 1.12	< 0.01
Dye visible (n = 44)	0.27 ± 0.18	
6 h		
Dye not visible (n = 65)	0.57 ± 0.57	NS*
Dye visible (n = 25)	0.41 ± 0.27	

* NS = not significant at the 0.05 level.

Table 4

Effect of Blood on Visibility of Dye in Suctioned Tracheobronchial Secretions*

Time†	Secretions		p Value
	Without Visible Blood (n = 156)	With Visible Blood (n = 114)	
2 h	90.2 (55/61)	3.4 (1/29)	< 0.001
4 h	79.6 (43/54)	2.8 (1/36)	< 0.001
6 h	61.0 (25/41)	0.0 (0/49)	< 0.001

*Values given as % of secretion with visible dye (No. of secretions with visible dye/total No. of secretions).

†At each time point, the visibility of dye was significantly greater for secretions without visible blood compared to secretions with visible blood.