

INTRODUCTION

- Sedated endoscopy for Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC) detection is invasive and expensive.
- Non-endoscopic BE/EAC detection tools have been guideline-endorsed to facilitate higher patient participation at lower cost.¹
- We previously described a promising panel of 5 methylated DNA markers (MDMs) assayed on esophageal specimens obtained by a sponge-on-a-string cell collection device in phase II studies.^{2,3}
- We aimed to train an algorithm (establish cutoff, to adjudicate samples as elevated/negative) using a final MDM panel followed by testing in an independent sample set.

METHODS

- Algorithm training samples (N=352) were prospectively collected from patients seen at 6 US medical centers. Test samples (N=125) were obtained from an independent, NIH-funded study conducted at 3 US medical centers. Both training and test sets were case control studies.
- Cases had endoscopic columnar metaplasia with histological intestinal metaplasia; controls had no endoscopic evidence of BE. Histology was reviewed by expert GI pathologists.
- The EsophaCap (Lucid, New York City, NY) cell collection device (25 mm, 10 pores per inch) was swallowed and withdrawn after 6-8 minutes followed by criterion standard endoscopy within 24 hours.
- DNA was extracted from collected cells and then bisulfite treated. Five MDMs were blindly assayed using the long probe quantitative amplified signal (LQAS) method.
- The algorithm was set using cross-validated logistic regression. The algorithm performance was evaluated with an independent test set.

RESULTS

- Baseline characteristics of patients in training and test sets are described in **Table 1**. Training and test sets were comparable.

Table 1 Baseline Characteristics of BE Cases and Controls

Variable	Training Set (N=198 controls, 154 cases)		Test Set (N= 44 controls, 81 cases)		P value
	Control	Case	Control	Case	
Mean age, (SD)	55 (13)	65 (10)	52 (15)	65 (11)	0.312
Male Sex (%)	102 (52)	119 (77)	17 (39)	64 (79)	0.992
Mean BMI (SD)	29 (7)	30 (6)	30 (7)	30 (6)	0.283
Ever Smokers (%)	77 (39)	87 (56)	18 (41)	47 (58)	0.733
Mean BE length, cm (SD)	-	4 (3)	-	5 (3)	0.070
Long segment BE, N, (%)	-	97 (63)	-	56 (69)	0.426
Short segment BE, N (%)	-	57 (37)	-	25 (31)	
BE dysplasia grade, N (%)					
- EAC	-	12 (8)	-	2 (2)	
- HGD	-	18 (12)	-	11 (14)	
- LGD	-	7 (4)	-	10 (12)	
- IND	-	18 (12)	-	14 (17)	
- NDBE (long segment)	-	57 (37)	-	25 (31)	
- NDBE (short segment)	-	42 (27)	-	19 (24)	

BE, Barrett's esophagus; BMI, body mass index; cm, centimeter; EAC, esophageal adenocarcinoma; HGD, high grade dysplasia; IND, indefinite for dysplasia; LGD, low grade dysplasia; NDBE, non-dysplastic Barrett's esophagus; SD, standard deviation.

- The final assay included 3 MDMs (*NDRG4*, *VAV3*, *ZNF682*) and a reference marker *B3GALT6*.
- Overall sensitivity for BE/EAC detection in the training set was 81% (68-94%) with specificity of 90% (79-98%). BE/EAC sensitivity in the test set was 88% (78-94%) at 84% (70-93%) specificity. Sensitivity for HGD/EAC was 100% in the training and test sets. Sensitivity for short segment NDBE in the test set was 63% (38-84%) (**Table 2**).

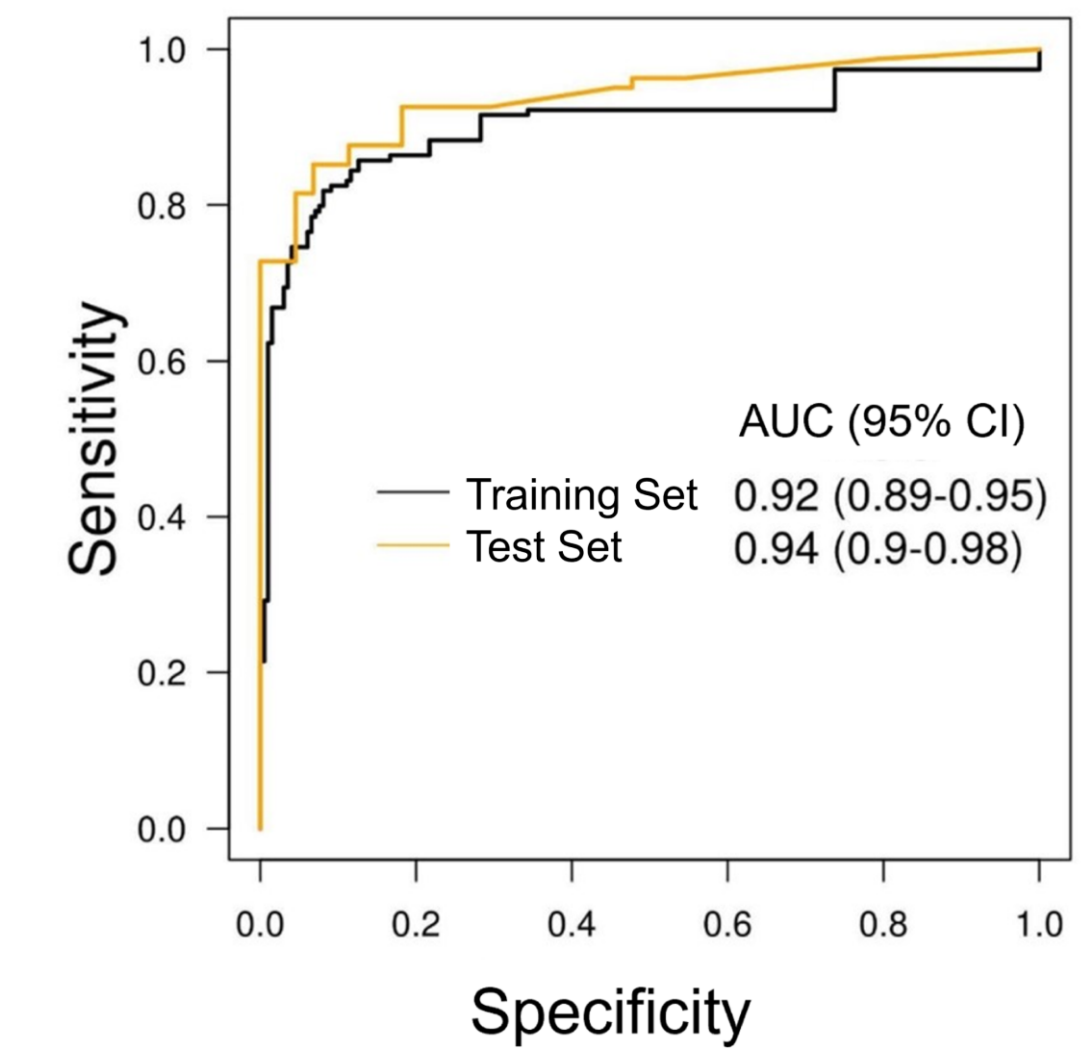
Table 2 Algorithm Performance in Training and Test Datasets

	Training Set		Test Set	
	n Positive	% Sensitivity (95% CI)	n Positive	% Sensitivity (95% CI)
Overall	125	81 (68-94)	71	88 (78-94)
- EAC	12	100 (100-100)	2	100 (16-100)
- HGD	18	100 (100-100)	11	100 (72-100)
- LGD	5	71 (0-100)	9	90 (55-100)
- IND	13	74 (0-100)	13	93 (66-100)
- NDBE (long segment)	52	91 (73-100)	24	96 (80-100)
- NDBE (short segment)	25	61 (25-100)	12	63 (38-84)
	n Positive	% Specificity (95% CI)	n Positive	% Specificity (95% CI)
Control (No BE)	20	90 (79-98)	7	84 (70-93)

BE, Barrett's esophagus; EAC, esophageal adenocarcinoma; HGD, high grade dysplasia; IND, indefinite for dysplasia; LGD, low grade dysplasia; NDBE, non-dysplastic Barrett's esophagus.

- Areas under the receiver operating characteristic (AUROC) curves for BE/EAC detection were 0.92 (95% CI 0.89-0.95) and 0.94 (0.90-0.98) in the training and test sets, respectively (Figure 1).

Figure 1. Area under the receiver operating characteristic curves in training and test sets.



- The algorithm was not influenced by age, sex, or smoking history.
- 97% of participants in the training set and 85% in the test set successfully swallowed the cell collection device, which was well tolerated and safe.

CONCLUSION

- A 3-MDM panel for BE/EAC detection demonstrated excellent sensitivity for high risk BE cases in multi-center case control training and test sets.
- The performance of this panel and algorithm will be validated in ongoing studies.

REFERENCES

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