Eosinophil Granule Major Basic Protein, Tissue Biopsy

CORRECTED

ARUP LABORATORIES

Patient: [redacted] DOB [redacted] Age: [redacted] Sex: [redacted] **Patient Identifiers:** [redacted] **Visit Number (FIN):** [redacted]	Client: [redacted] Physician: [redacted]	ARUP Test Code: 2010921 Collection Date: 06/21/2022 Received in lab: 06/22/2022 Completion Date: 06/22/2022

Immunodermatology Eosinophil Granule Major Basic Protein 1 Tissue Test Report Navigation Guide

The Immunodermatology TESTING REPORT from the University of Utah follows "See Note" and is arranged as outlined below on the following pages:

CLINICAL INFORMATION
This content is provided by the ordering clinician and includes the indications for testing.

Specimen Details
This includes specimen identification, clinician-provided body location, procurement information and date, and laboratory received date.

DIAGNOSTIC INTERPRETATION
This is a synopsis of key test findings and their diagnostic relevance.

RESULTS
This section reports discrete findings including presence of intact eosinophils and maximal counts per high power field, description of the extracellular eosinophil granule protein deposition pattern graded on a visual analog scale, and percentage estimate of positive tissue staining.

COMMENTS
Specific

These comments provide an explanation of the test results as they relate to clinical considerations and may include recommendations to correlate with other testing. If prior eosinophil granule protein testing has been performed, this section includes a summary chart of previous and current findings from testing ordered by the same clinician client.

General

These comments present information about the test(s) and the component(s) assessed to aid interpretation of clinical applicability.

TESTING METHODS
The section summarizes the procedures performed, the interpretation schema, and the applicable laboratory-developed test disclaimer.

RESULT IMAGES (included and available when applicable)
Images of representative positive, unusual, and/or special interest findings* are displayed in this section, which may be found on the next page. High resolution, color digital files of the images may be requested by contacting ARUP Client Services at 1-800-242-2787, option 2, and ask to speak with the Immunodermatology Laboratory at the University of Utah regarding patient results.

* Note that immunofluorescence-stained tissue slides are not kept longer than two weeks after testing because of degradation in findings; therefore, additional images cannot be acquired after that time without retesting.

Biopsy site and adequate tissue are critical for accurate diagnostic findings. For additional information, refer to:
arupconsult.com/content/eosinophil-associated-diseases

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Jonathan R. Genzen, MD, PhD, Laboratory Director

Chart continues on following page(s)

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IMMUNODERMATOLOGY LABORATORY REPORT

****** Amended ******

[redacted]

Amended Report: EMBP1 TIS - Lab Error

Submitter

ARUP Sendouts

Eosinophil Granule Major Basic Protein, Tissue Biopsy (Edited)

Specimen Source
Mucosa

Amended Report: this section has been changed.

TESTING REPORT follows "See Note"
See Note

CLINICAL INFORMATION
Eosinophilic esophagitis, eosinophilic gastrointestinal disease (EGID)

Specimen Details
B22-00862 A - Esophagus, proximal; Collected: 6/21/2022; Received: 6/22/2022
B22-00862 B - Duodenum, bulb; Collected: 6/21/2022; Received: 6/22/2022

Original Report

DIAGNOSTIC INTERPRETATION

Positive cellular and extracellular eosinophil granule major basic protein 1 (eMBP1), abnormally increased cells in proximal esophagus

[redacted]
PCP: Unspecified

(Specimen A) with relatively intense extracellular distribution along one edge, and likely abnormally distributed positive staining in duodenum (Specimen B); the findings in the esophagus (Specimen A) provide support for the diagnosis of eosinophilic esophagitis or other eosinophil-related inflammation, and the findings in duodenum further suggest eosinophilic gastrointestinal disease (EGID)

Esophagus, proximal (Specimen A)
Overall grade, 2+
Approximate tissue area with staining, 35 percent

Duodenum, bulb (Specimen B)
Overall grade, 2-3+
Approximate tissue area with staining, 50 percent

(See Results, Comments, and Previous and Current Test Results Summary Chart)

RESULTS
EOSINOPHIL MAJOR BASIC PROTEIN 1 TESTING

Examination of the tissue sections from proximal esophagus (Specimen A) and duodenal bulb (Specimen B) tested for eosinophil granule major basic protein 1 (eMBP1) reveals:

Specimen A	Cellular*: 3+ intensity, 2+ extent (Eosinophil count, 23 per high power field, 400x)
	Extracellular: 2-3+ intensity, 2+ extent with with patchy interstitial granules and patchy confluent tissue
Specimen B	Cellular*: 3+ intensity, 2-3+ extent (Eosinophil count, 36 per high power field, 400x)
	Extracellular: 2-3+ intensity, 2-3+ extent with patchy confluent tissue and patchy granules

* Intact cells showing positive eMBP1 staining counted per 400x (40x objective lens and 10x eyepiece lens) high power field (HPF) in areas of sections with maximal cells. Some cells may not be counted as intact cells that are obfuscated by extracellular eMBP1 deposition, and some degranulated cells that appear mainly intact may be included.

COMMENTS
Specific

[redacted]
PCP: Unspecified

The results from this testing for eosinophil granule major basic protein 1 (eMBP1) show abnormal increased infiltration of eosinophils and extracellular eMBP1 deposition in the proximal esophagus tissues (Specimen A), implicating involvement of eosinophils in the pathogenic activity. The extracellular deposition is out-of-proportion to the infiltrating eosinophils in amount and distribution in patchy areas. The positive findings in the proximal esophagus tissues support the diagnosis of eosinophilic esophagitis or other eosinophil-related inflammation. The cell numbers in these tissues are sufficient to constitute a criterion for the diagnosis of eosinophilic esophagitis; extracellular eMBP1 deposition is not established as a diagnostic feature but often is observed in the disorder.

The results from this testing for eMBP1 in the duodenal bulb tissues (Specimen B) are positive but indeterminate for being abnormal and the degree of positivity relative to normal. As judged by the staining, eosinophil activity, as a contributor to the pathophysiology, does not appear to be prominently increased, although the distribution of extracellular eMBP1, including the confluent tissue eMBP1 deposition, likely is abnormal, reflecting local ongoing or recent previous eosinophil activity. The cell numbers should be compared to known normal numbers in this tissue area; of note, the numbers may not account for cells that have infiltrated and degranulated, and, therefore, may be spuriously low. Moreover, the findings should be considered in view of the size and fragmentation of the tissues. Small tissue specimens are more prone to crush artifact in procurement and freeze artifact in processing. Other considerations are that the findings in the tissues could be a remnant of a previous state with greater eosinophil involvement and do not exclude the possibility of more prominent involvement elsewhere. Patients with eosinophilic esophagitis often show prominent eMBP1 immunostaining in small bowel tissues, but this is not a defined diagnostic criterion for eosinophilic esophagitis.

See chart (below) for summary of previous and current eosinophil granule protein testing results. Note that the findings may not be directly comparable because of variability in the location from which the tissue biopsies have been obtained and the architecture of the specimens, as well as the often patchy nature of eosinophil inflammation in the gastrointestinal tract.

Correlation of the findings with clinical presentation is needed, including with respect to treatment status. Correlation with histopathological examination of formalin-fixed tissue may be helpful, although extracellular granule protein deposition and degranulated cells may not be recognized in formalin-fixed tissues.

High resolution, color digital images of representative direct immunofluorescence findings are available for this testing (see images in the Enhanced Electronic Report/EELR). If you would like a

hard copy or an electronic file of the images and/or if it would be helpful to discuss the patient case with this report, contact ARUP Client Services at 1-800-242-2787, option 2, and ask to speak with the Immunodermatology Laboratory at the University of Utah regarding patient results.

General

Eosinophil infiltration and/or degranulation normally are present in thymus, lymph node, gastrointestinal tract from stomach through large intestine, and bone marrow; therefore, cellular and extracellular eosinophil granule major basic protein 1 (eMBP1) immunostaining normally is positive in specimens from the duodenum but normally is negative in esophagus. In normal gastrointestinal tract tissues from small bowel, eosinophils showing positive eMBP1 staining tend to be clustered near the muscularis mucosa with positive extracellular granules dispersed around and among the positive cellular staining.

Eosinophil granule proteins, including eMBP1, have various and numerous toxic effects on tissues and organs. In determining whether eosinophils and eosinophil granule proteins may be playing a pathogenic role, consideration must be given to the treatment status of the patient (glucocorticoid and other therapies may rapidly reduce eosinophils in tissues as well as blood) and whether the specimens are representative of involved tissues (active eosinophil inflammation of gastrointestinal tissues may be patchy). Extracellular eosinophil granule proteins may persist in tissues for a long time after deposition and may not reflect current activity. Moreover, some positive staining likely is the result of crush artifact in the specimen procurement and freeze artifact in processing, especially extracellular granules in areas where eosinophils normally are present and/or infiltrate. Crush artifact may be prominent in small specimens. Also, some eosinophils may be observed in tissues from incidental intravascular presence, more common in patients with peripheral blood eosinophilia and more common in highly vascularized tissues.

TESTING METHODS

The specimens from esophagus, proximal (Specimen A) and duodenum, bulb (Specimen B) received in Michel transport medium, after washing and cryoembedding, are sectioned. Sections from each specimen are reacted with antibody to eosinophil granule major basic protein 1 (eMBP1) by indirect immunofluorescence, utilizing a fluorescein isothiocyanate (FITC)-conjugated secondary antibody for detection, and subsequently examined by fluorescence microscopy to identify intact eosinophils and extracellular eosinophil granule protein deposition. The antibody-stained sections are graded on a visual analog scale with reference images. In addition to the overall grade recorded for cellular and extracellular staining in each specimen, a maximal eosinophil count per high power field, 400x, is performed,

[redacted]
PCP: Unspecified

and an estimate of the percentage of tissue with positive eMBP1 staining is rendered. The technically adequate hematoxylin and eosin (H and E)-stained section of the tissue is comparatively examined for morphological features and orientation. The antibody-stained sections also are compared to serial sections stained with normal rabbit IgG (as a negative control). A skin biopsy specimen with multiple infiltrating eosinophils and extracellular eMBP1 deposition serves to establish that the expected specific staining is detected in the assay (as a positive control). This indirect immunofluorescence testing was developed and its performance characteristics determined by the Immunodermatology Laboratory at the University of Utah. It has not been cleared or approved by the FDA (US Food and Drug Administration). FDA clearance or approval currently is not required for clinical use of this testing performed in a CLIA-certified laboratory (Clinical Laboratory Improvement Amendments). The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

Electronically signed by [redacted], MD, on 06/22/22 at 11:32 AM.

CLINICAL INFORMATION
Eosinophilic esophagitis, eosinophilic gastrointestinal disease (EGID)

Specimen Details
B22-00862 A - Esophagus, proximal; Collected: 6/21/2022; Received: 6/22/2022
B22-00862 B - Duodenum, bulb; Collected: 6/21/2022; Received: 6/22/2022

Revised Report

DIAGNOSTIC INTERPRETATION

** Report revised with additional information from retesting with diminished evidence of eosinophil involvement **

Approximately normal cellular and extracellular eosinophil granule major basic protein 1 (eMBP1), in esophagus (Specimen A) with relatively intense extracellular distribution along one edge, and likely abnormally distributed positive staining in duodenum (Specimen B); the findings in the esophagus (Specimen A) do not provide support for the diagnosis of eosinophilic esophagitis or other eosinophil-related inflammation, and the findings in duodenum further are indeterminate for eosinophilic gastrointestinal disease (EGID)

Esophagus (Specimen A)

[redacted]
PCP: Unspecified

Overall grade, 1+
Approximate tissue area with staining, 15 percent

Duodenum, bulb (Specimen B)
Overall grade, 2+
Approximate tissue area with staining, 35 percent

(See Results, Comments, and Previous and Current Test Results Summary Chart)

RESULTS
EOSINOPHIL MAJOR BASIC PROTEIN 1 TESTING

Examination of the tissue sections from proximal esophagus (Specimen A) and duodenal bulb (Specimen B) tested for eosinophil granule major basic protein 1 (eMBP1) reveals:

Specimen A	Cellular*: 3+ intensity, 1+ extent (Eosinophil count, 9 per high power field, 400x)
	Extracellular: 2-3+ intensity, 1+ extent with with patchy interstitial granules and patchy confluent tissue
Specimen B	Cellular*: 3+ intensity, 2-3+ extent (Eosinophil count, 18 per high power field, 400x)
	Extracellular: 2-3+ intensity, 2+ extent with patchy confluent tissue and patchy granules

* Intact cells showing positive eMBP1 staining counted per 400x (40x objective lens and 10x eyepiece lens) high power field (HPF) in areas of sections with maximal cells. Some cells may not be counted as intact cells that are obfuscated by extracellular eMBP1 deposition, and some degranulated cells that appear mainly intact may be included.

COMMENTS
Specific

The results from this retesting for eosinophil granule major basic protein 1 (eMBP1) show abnormal increased infiltration of eosinophils and extracellular eMBP1 deposition in the esophagus tissues (Specimen A), implicating involvement of eosinophils in the pathogenic activity but less than previously observed and likely more accurately reflecting eosinophil involvement. The extracellular deposition is proportional to the infiltrating eosinophils in amount

[redacted]
PCP: Unspecified

and distribution in patchy areas. The positive findings in the esophagus tissues are insufficient to support the diagnosis of eosinophilic esophagitis or other eosinophil-related inflammation. The cell numbers in these tissues are insufficient to constitute a criterion for the diagnosis of eosinophilic esophagitis; extracellular eMBP1 deposition is not established as a diagnostic feature but often is observed in the disorder.

The results from this testing for eMBP1 in the duodenal bulb tissues (Specimen B) are positive but indeterminate for being abnormal and the degree of positivity relative to normal. As judged by the staining, eosinophil activity, as a contributor to the pathophysiology, does not appear to be prominently increased, although the distribution of extracellular eMBP1, including the confluent tissue eMBP1 deposition, likely is abnormal, reflecting local ongoing or recent previous eosinophil activity. The cell numbers should be compared to known normal numbers in this tissue area; of note, the numbers may not account for cells that have infiltrated and degranulated, and, therefore, may be spuriously low. Moreover, the findings should be considered in view of the size and fragmentation of the tissues. Small tissue specimens are more prone to crush artifact in procurement and freeze artifact in processing. Other considerations are that the findings in the tissues could be a remnant of a previous state with greater eosinophil involvement and do not exclude the possibility of more prominent involvement elsewhere. Patients with eosinophilic esophagitis often show prominent eMBP1 immunostaining in small bowel tissues, but this is not a defined diagnostic criterion for eosinophilic esophagitis.

See chart (below) for summary of previous and current eosinophil granule protein testing results. Note that the findings may not be directly comparable because of variability in the location from which the tissue biopsies have been obtained and the architecture of the specimens, as well as the often patchy nature of eosinophil inflammation in the gastrointestinal tract.

Correlation of the findings with clinical presentation is needed, including with respect to treatment status. Correlation with histopathological examination of formalin-fixed tissue may be helpful, although extracellular granule protein deposition and degranulated cells may not be recognized in formalin-fixed tissues.

High resolution, color digital images of representative direct immunofluorescence findings are available for this testing (see images in the Enhanced Electronic Report/EELR). If you would like a hard copy or an electronic file of the images and/or if it would be helpful to discuss the patient case with this report, contact ARUP Client Services at 1-800-242-2787, option 2, and ask to speak with the Immunodermatology Laboratory at the University of Utah regarding patient results.

General

[redacted]
PCP: Unspecified

Eosinophil infiltration and/or degranulation normally are present in thymus, lymph node, gastrointestinal tract from stomach through large intestine, and bone marrow; therefore, cellular and extracellular eosinophil granule major basic protein 1 (eMBP1) immunostaining normally is positive in specimens from the duodenum but normally is negative in esophagus. In normal gastrointestinal tract tissues from small bowel, eosinophils showing positive eMBP1 staining tend to be clustered near the muscularis mucosa with positive extracellular granules dispersed around and among the positive cellular staining.

Eosinophil granule proteins, including eMBP1, have various and numerous toxic effects on tissues and organs. In determining whether eosinophils and eosinophil granule proteins may be playing a pathogenic role, consideration must be given to the treatment status of the patient (glucocorticoid and other therapies may rapidly reduce eosinophils in tissues as well as blood) and whether the specimens are representative of involved tissues (active eosinophil inflammation of gastrointestinal tissues may be patchy). Extracellular eosinophil granule proteins may persist in tissues for a long time after deposition and may not reflect current activity. Moreover, some positive staining likely is the result of crush artifact in the specimen procurement and freeze artifact in processing, especially extracellular granules in areas where eosinophils normally are present and/or infiltrate. Crush artifact may be prominent in small specimens. Also, some eosinophils may be observed in tissues from incidental intravascular presence, more common in patients with peripheral blood eosinophilia and more common in highly vascularized tissues.

Eosinophil Granule Protein Localization

Specimens		eMBP1, eosinophil major basic protein 1, staining grade and cell count*					
		Small Bowel Duodenum		Stomach		Esophagus	
Specimen Number	Date of Specimen	Grade	Cell Count	Grade	Cell Count	Grade	Cell Count
21-0001	05/04/21	2+	40	2+	20	1+	15
22-0001	01/03/22	2+	18	NB		1+	9

Chart Key:

NB = Not Biopsied

* The tissue sections tested for eMBP1 by indirect immunofluorescence are graded on a visual analog scale with

[redacted]
PCP: Unspecified

reference images. Intact cells showing positive eMBP1 staining are counted per 400x (40x objective lens and 10x eyepiece lens) high power field (HPF) in areas of maximal cells.

TESTING METHODS
The specimens from esophagus (Specimen A) and duodenum, bulb (Specimen B) received in Michel transport medium, after washing and cryoembedding, are sectioned. Sections from each specimen are reacted with antibody to eosinophil granule major basic protein 1 (eMBP1) by indirect immunofluorescence, utilizing a fluorescein isothiocyanate (FITC)-conjugated secondary antibody for detection, and subsequently examined by fluorescence microscopy to identify intact eosinophils and extracellular eosinophil granule protein deposition. The antibody-stained sections are graded on a visual analog scale with reference images. In addition to the overall grade recorded for cellular and extracellular staining in each specimen, a maximal eosinophil count per high power field, 400x, is performed, and an estimate of the percentage of tissue with positive eMBP1 staining is rendered. The technically adequate hematoxylin and eosin (H and E)-stained section of the tissue is comparatively examined for morphological features and orientation. The antibody-stained sections also are compared to serial sections stained with normal rabbit IgG (as a negative control). A skin biopsy specimen with multiple infiltrating eosinophils and extracellular eMBP1 deposition serves to establish that the expected specific staining is detected in the assay (as a positive control). This indirect immunofluorescence testing was developed and its performance characteristics determined by the Immunodermatology Laboratory at the University of Utah. It has not been cleared or approved by the FDA (US Food and Drug Administration). FDA clearance or approval currently is not required for clinical use of this testing performed in a CLIA-certified laboratory (Clinical Laboratory Improvement Amendments). The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

Electronically signed by [redacted], MD, on 06/24/22 at 12:13 AM.
Amended Report: this section has been changed.




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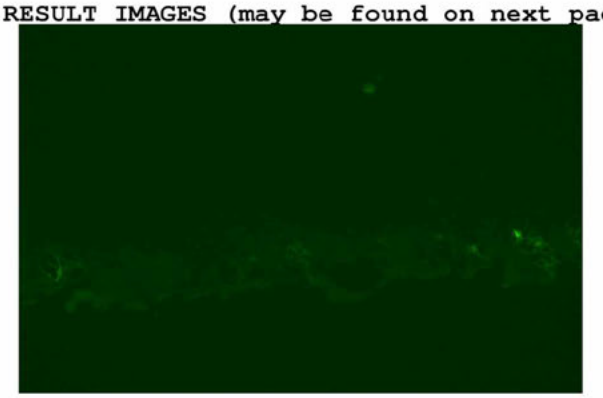
Chart continues on following page(s)

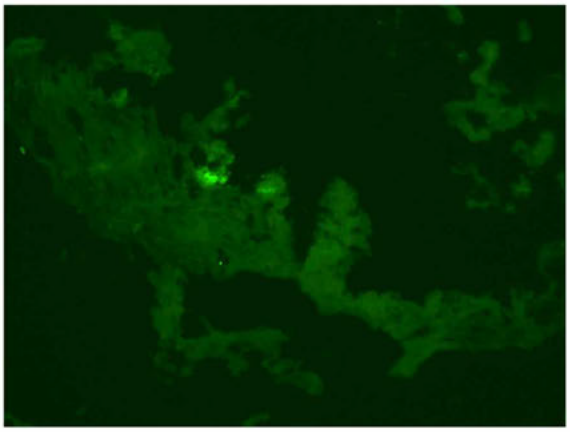
[redacted]

PCP: Unspecified

RESULT IMAGES (may be found on next page)

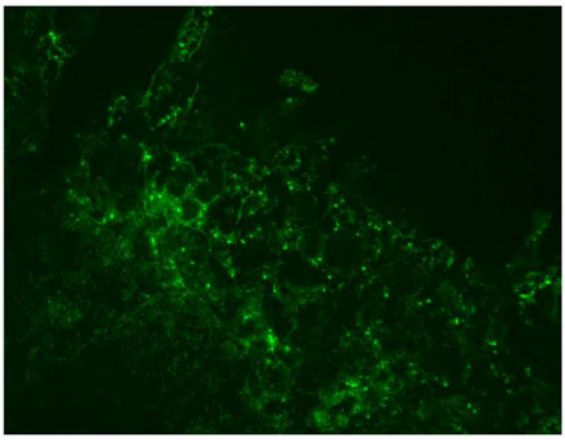


Proximal esophagus (Specimen A), eMBP1, 50x

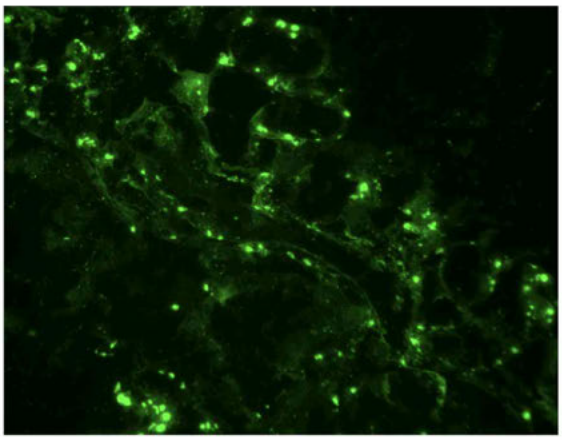


Proximal esophagus (Specimen A), eMBP1, 100x

[redacted]
PCP: Unspecified



Duodenal bulb (Specimen B), eMBP1, 50x



Duodenal bulb (Specimen B), eMBP1, 100x

Amended Report: this section has been changed.

Resulting Laboratory

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