CMS Crosswalk/Gapfill Meeting

June 22, 2020

Ambry Genetics PLA Codes 0101U-0103U, 0129U, 0157U-0162U

Summary of PLA Codes 0101U-0103U, 0129U

Code	Name	Gene SEQ	Gene DUP DEL	PLUS:
0101U	**ColoNext**	**15**	**17**	
0102U	**BreastNext**	**17**	**17**	**Sanger**
				MLPA
0103U	**OvaNext**	**24**	**25**	**aCGH**
0129U	**BRCAPlus**	**8**	**8**	

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Ambry Genetics, Dr. Brigette Tippin Davis

1. Mu W, *et al.* Sanger confirmation is required to achieve optimal sensitivity and specificity in next-generation sequencing panel testing. J Mol Diagn. 2016. 18(6):923-932.

Features of PLA Codes 0101U-0103U, 0129U

Test	Rationale for Gapfill Request
Sanger	Performed for complex genomic regions, including: repeat regions, GC-rich regions and for any regions missing or with insufficient read depth coverage for reliable heterozygous variant detection. Reportable small insertions and deletions, potentially homozygous variants, variants in regions complicated by pseudogene interference, and single nucleotide variant calls not satisfying 100x depth of coverage and 35% het ratio thresholds are verified by Sanger sequencing.
MLPA	Performed for the pseudogene region of PMS2 due to number of pseudogene copies and sequence similarity across the genome. If a deletion is detected in exons 13, 14, or 15 of *PMS2*, double stranded sequencing of the appropriate exon(s) of the pseudogene, *PMS2CL*, will be performed to determine if the deletion is located in the *PMS2* gene or pseudogene.
aCGH	Gross deletion/duplication analysis is performed for the covered exons and untranslated regions of all 17 genes using read-depth from NGS data with confirmatory multiplex ligation-dependent probe amplification **(MLPA)** and/or targeted chromosomal microarray.

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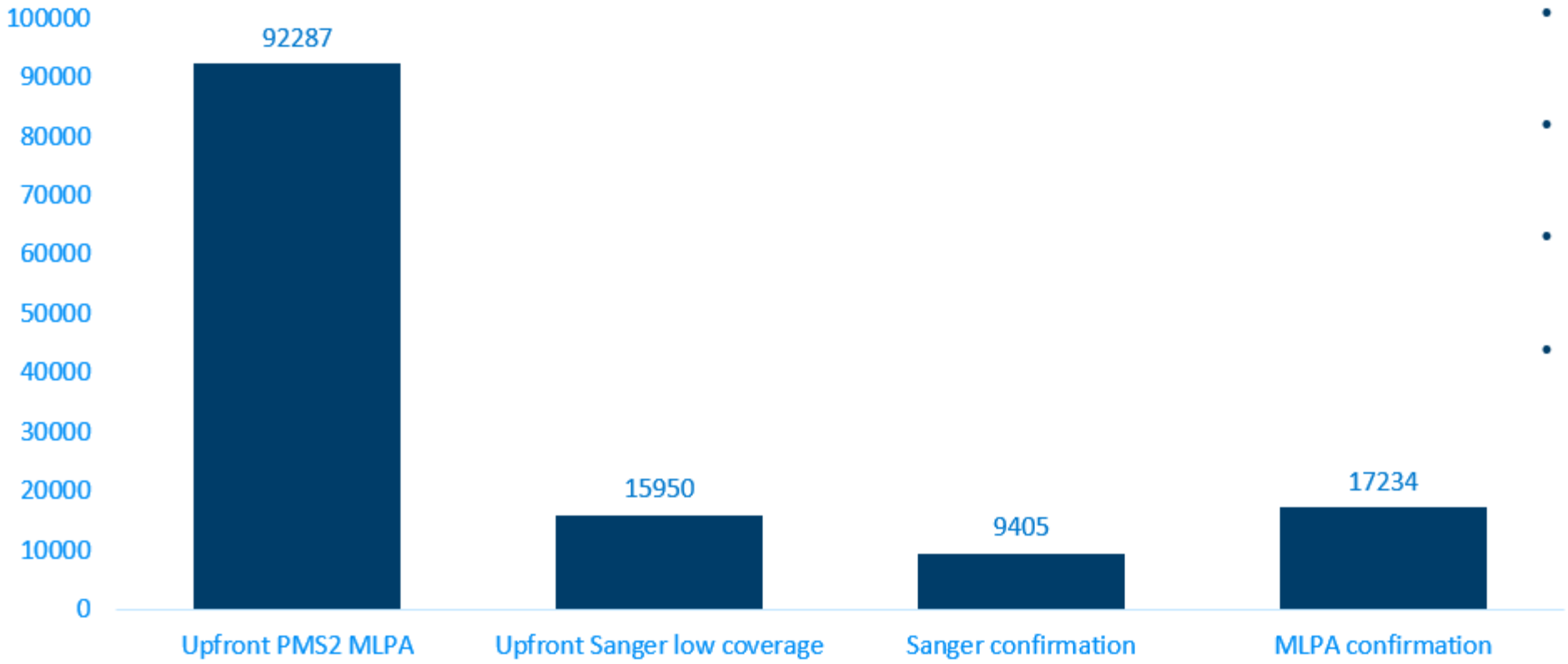
Features of PLA Codes 0101U-0103U, 0129U

Additional methodology performed for 107,258 cancer panels in 2019



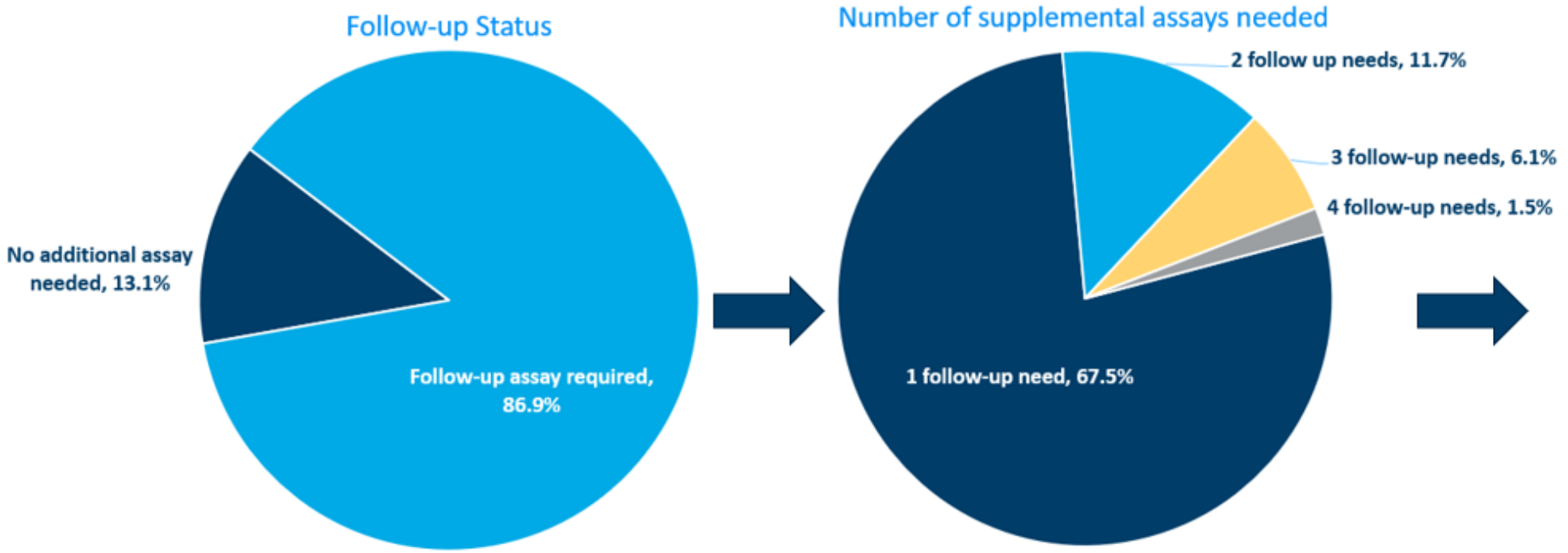
Legend

- Upfront *PMS2* MLPA = All test orders including PMS2 require MLPA on the front end
- Upfront Sanger low coverage = Loci with <20x NGS coverage that require Sanger fill in
- Sanger confirmation = Orthogonal confirmation of a SNV call from the NGS pipeline
- MLPA confirmation = Orthogonal confirmation of a del/dup call from the NGS pipeline

All counts are on the procedure level

Features of PLA Codes 0101U-0103U, 0129U

Impact of additional methodologies performed



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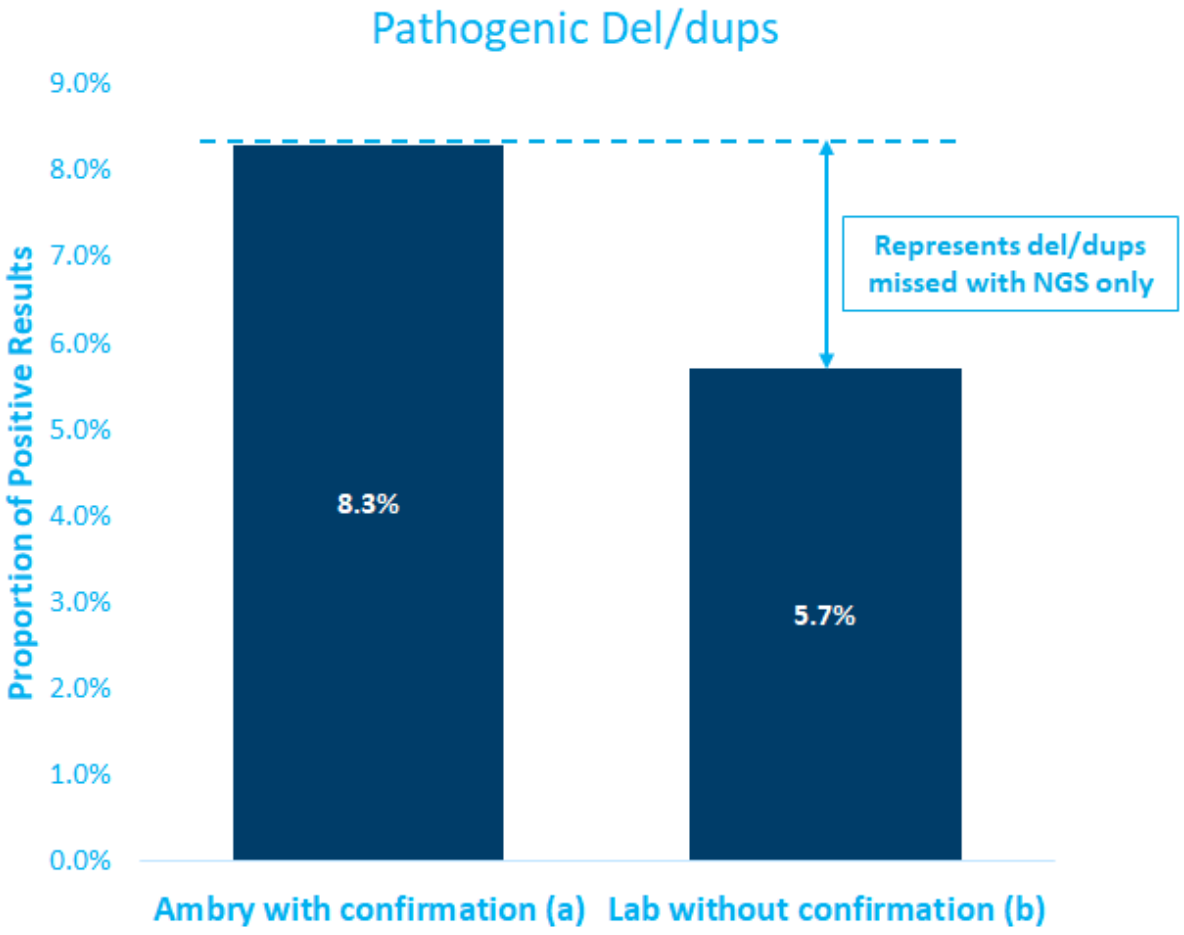
Features of PLA Codes 0101U-0103U, 0129U

Orthogonal confirmation of gross del/dups increases sensitivity

- Performing orthogonal confirmation of del/dups allows for a highly sensitive NGS pipeline.
- Without confirmation, NGS del/dup thresholds must be more stringent to avoid false positives and results in missed true positives.



a) LaDuca H. et al. *Genet Med* 22, 407–415 (2020).
b) Neben CL et al. *J Mol Diagn*. 2019 Jun 10.

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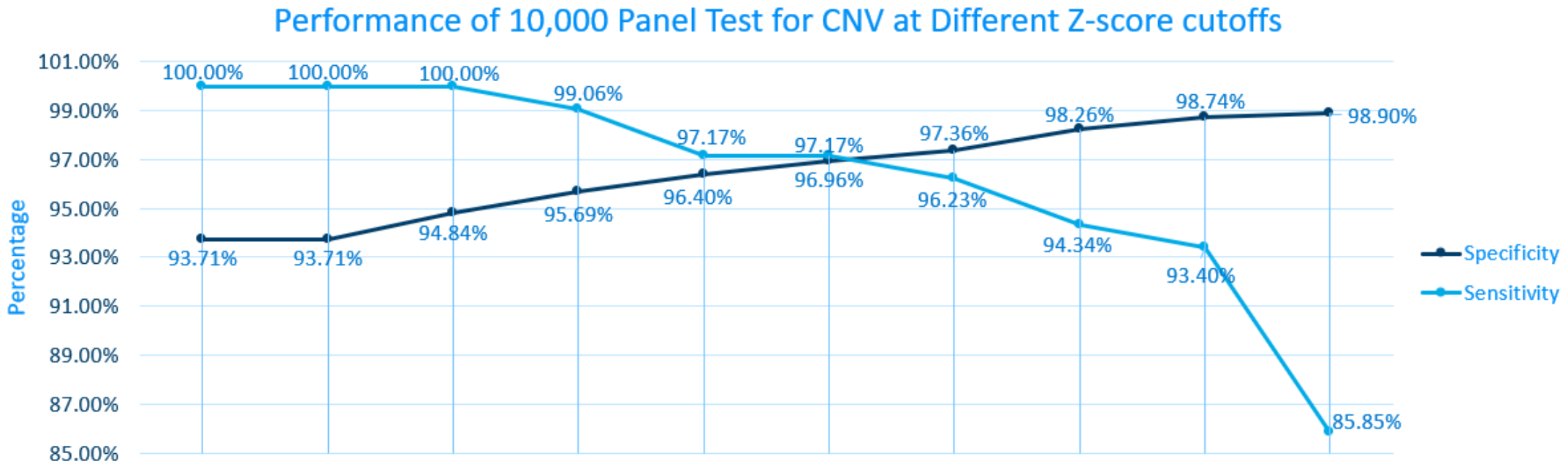
Features of PLA Codes 0101U-0103U, 0129U

Additional methodology performed for 107,258 cancer panels in 2019



z-score cutoff	4.5	< 5.0	< 5.1	< 5.2	< 5.3	< 5.4	< 5.5	< 6.0	< 7.0	< 8.0
True Positive	106	106	106	105	103	103	102	100	99	91
False Negative	0	0	0	1	3	3	4	6	7	15
False Positive	622	622	511	426	356	301	261	172	125	109
True Negative	9272	9272	9383	9468	9538	9593	9633	9722	9769	9785
Specificity	93.71%	93.71%	94.84%	95.69%	96.40%	96.96%	97.36%	98.26%	98.74%	98.90%
Sensitivity	100.00%	100.00%	100.00%	99.06%	97.17%	97.17%	96.23%	94.34%	93.40%	85.85%

Eliminating orthogonal confirmation of del/dups and maintaining 100% sensitivity would result in 511 false positives per 10,000 cases

PLA Codes 0101U-0103U, 0129U

Many commercial laboratories do not perform additional procedures that are performed as part of the above PLAs.

Sample report from national commercial lab

Limitations

This test aims to detect all clinically relevant variants within the genes analyzed (defined above). The majority of these genes are assessed for variants within all coding exons (and adjacent intronic sequence). Exons 12-15 of *PMS2* cannot be reliably assessed with standard target enrichment protocols. For the *CDK4*, *MITF*, *POLD1* and *POLE* genes, the elevated risk of cancer is associated with distinct functional genomic regions, therefore, the complete coding sequences of these genes are not reported, but instead only the following regions: *CDK4* - chr12:g.58145429-58145431 (codon 24), *MITF* - chr3:g.70014091 (including c.952G>A), *POLD1* - chr19:g.50909713 (including c.1433G>A) and *POLE* - chr12:g.133250250 (including c.1270C>G). In *EPCAM*, only large deletions and duplications including the 3' end of the gene are reported since these are the only variants known to silence the *MSH2* gene and therefore increase risk of

PLA Codes 0101U & 0102U

Code	Full Text	Public Comment	Rationale
0101U ColoNext	Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with mRNA analytics to resolve variants of unknown significance when indicated (15 genes [sequencing and deletion/duplication], EPCAM and GREM1 [deletion/duplication only])	Appealed 2019 crosswalk recommendation. For 2020, requesting Gapfill for component codes plus additional procedures for non-GSP molecular analysis for comparable resources and work for special variant of unknown significance analytics	Test includes DNA Full Gene Sequencing and deletion/duplication. PLUS additional procedures: MLPA for PMS2, aCGH for del/dup and Sanger for low quality reads as indicated
0102U BreastNext	Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with mRNA analytics to resolve variants of unknown significance when indicated (17 genes [sequencing and deletion/duplication])	Appealed 2019 crosswalk recommendation. For 2020, requesting Gapfill for component codes plus additional procedures for non-GSP molecular analysis for comparable resources and work for special variant of unknown significance analytics	Test includes DNA Full Gene Sequencing and deletion/duplication. PLUS additional procedures: MLPA for PMS2, aCGH for del/dup and Sanger for low quality reads as indicated

PLA Codes 0103U & 0129U

Code	Full Text	Public Comment	Rationale
0103U OvaNext	Hereditary ovarian cancer (eg, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with mRNA analytics to resolve variants of unknown significance when indicated (24 genes [sequencing and deletion/duplication], EPCAM [deletion/duplication only])	Appealed 2019 crosswalk recommendation. For 2020, requesting Gapfill for component codes plus additional procedures for non-GSP molecular analysis for comparable resources and work for special variant of unknown significance analytics	Test includes DNA Full Gene Sequencing and deletion/duplication. PLUS additional procedures: MLPA for PMS2, aCGH for del/dup and Sanger for low quality reads as indicated
0129U BRCAPlus	Hereditary breast cancer–related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis and deletion/duplication analysis panel (ATM, BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, and TP53)	Appealed 2019 crosswalk recommendation. For 2020, requesting Gapfill for component codes plus additional procedures for non-GSP molecular analysis for comparable resources and work for special variant of unknown significance analytics	Test includes DNA Full Gene Sequencing and deletion/duplication. PLUS additional procedures: MLPA or aCGH for del/dup and Sanger for low quality reads as indicated

PLA Codes 0157U - 0162U
+RNAInsight Process is **Independent and Parallel**

DNA Lab Process

→ Lab A

- Blood DNA Extraction (EDTA Tube)
- DNA Library Prep
- DNA Capture
- Sequencing (# of Exons sequenced - see next slide)
- Data Analysis

RNA Lab Process

→ Lab B

- Blood RNA Extraction (Paxgene Tube)
- cDNA Library Prep
- cDNA Capture
- Sequencing (# of Exons sequenced - see next slide)
- Data Analysis

Integrated results/report

PLA Codes 0157U - 0162U
RNA Exon Count Mirrors DNA Exon Count

Gene	# Coding Exons (DNA)	# Total Exons (DNA)	# Coding Exons (RNA)
APC	15	**16**	15
MLH1	19	**19**	19
MSH2	16	**16**	16
MSH6	10	**10**	10
PMS2	15	**15**	15

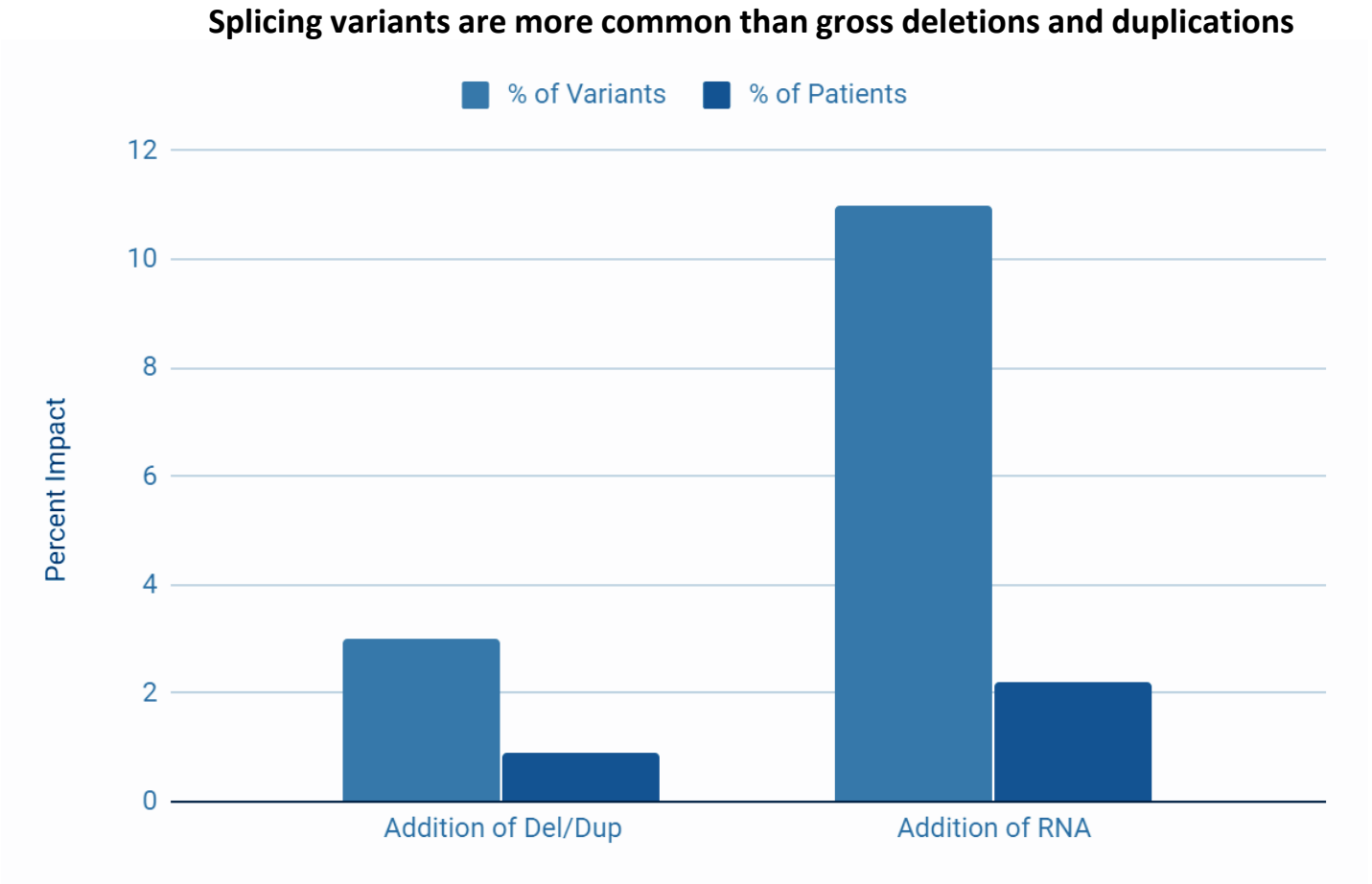
PLA Codes 0157U - 0162U
Impact of +RNAInsight is **> Del/Dup Analysis**

More Accurately Clarify New Variants

Reclassify VUS

Detect previously unidentified deep-intronic variants

11% of Variants



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PLA Codes +0157U - +0162U

Code	Full Text	Public Comment	Rationale
+0157U APC +RNA Insight	APC (APC regulator of WNT signaling pathway) (eg, familial adenomatosis polyposis [FAP]). **RNA analysis to screen for abnormal RNA transcripts and to resolve DNA variants of unknown significance when indicated [1 gene].** **(Use 0157U in conjunction with 81201)**	Crosswalk 81201/NLA = **$780.00**	Test includes similar gene content, lab process and resources as 81201
+0158U MLH1 +RNA Insight	MLH1 (mutL homolog 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome). **RNA analysis to screen for abnormal RNA transcripts and to resolve DNA variants of unknown significance when indicated [1 gene].** **(Use 0158U in conjunction with 81292)**	Crosswalk 81292/NLA = **$675.40**	Test includes similar gene content, lab process and resources as 81292
+0159U MSH2 +RNA Insight	MSH2 (mutS homolog 2) (eg, hereditary colon cancer, Lynch syndrome) **RNA analysis to screen for abnormal RNA transcripts and to resolve DNA variants of unknown significance when indicated [1 gene].** **(Use 0159U in conjunction with 81295)**	Crosswalk 81295/NLA = **$381.70**	Test includes similar gene content, lab process and resources as 81295

PLA Codes 0157U - 0162U

Code	Full Text	Public Comment	Rationale
+0160U MSH6 +RNA Insight	MSH6 (mutS homolog 6) (eg, hereditary colon cancer, Lynch syndrome). **RNA analysis to screen for abnormal RNA transcripts and to resolve DNA variants of unknown significance when indicated [1 gene).** **(Use 0160U in conjunction with 81298)**	Crosswalk 81298/NLA = **$641.85**	Test includes similar gene content, lab process and resources as 81298
+0161U PMS2 +RNA Insight	PMS2 (PMS1 homolog 2, mismatch repair system component) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome). **RNA analysis to screen for abnormal RNA transcripts and to resolve DNA variants of unknown significance when indicated [1 gene].** **(Use 0161U in conjunction with 81317)**	Crosswalk 81317/NLA = **$676.50**	Test includes similar gene content, lab process and resources as 81317
+0162U Lynch +RNA Insight	Hereditary colon cancer disorders (eg, Lynch syndrome) **RNA analysis to screen for abnormal RNA transcripts and to resolve DNA variants of unknown significance when indicated [4 genes].** **(Use 0162U in conjunction with 81292, 81295, 81298, 81317, 81435)**	Crosswalk 81435/NLA = **$584.90**	Test includes similar gene content, lab process and resources as 81435

THANK YOU

CMS Annual Lab Meeting, June 24, 2019
Ambry Genetics, Dr. Brigette Tippin Davis

SLIDES FOR REFERENCE

CMS Annual Lab Meeting, June 22, 2020
Ambry Genetics, Dr. Brigette Tippin Davis

PLA Code 0101U

Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH to resolve variants of unknown significance when indicated (15 genes [sequencing and deletion/duplication], EPCAM and GREM1 [deletion/duplication only])

Public Comment	Rationale
Appealed 2019 crosswalk recommendation. For 2020, requesting Gapfill for component codes plus additional procedures for non-GSP molecular analysis for comparable resources and work for special variant of unknown significance analytics	Test includes DNA Full Gene Sequencing and deletion/duplication. PLUS additional procedures: MLPA for PMS2, aCGH for del/dup and Sanger for low quality reads as indicated

PLA Code 0102U

Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated (17 genes [sequencing and deletion/duplication])

Public Comment	Rationale
Appealed 2019 crosswalk recommendation. For 2020, requesting Gapfill for component codes plus additional procedures for non-GSP molecular analysis for comparable resources and work for special variant of unknown significance analytics	Test includes DNA Full Gene Sequencing and deletion/duplication. PLUS additional procedures: MLPA for PMS2, aCGH for del/dup and Sanger for low quality reads as indicated

PLA Code 0103U

Hereditary ovarian cancer (eg, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated (24 genes [sequencing and deletion/duplication], EPCAM [deletion/duplication only])

Public Comment	Rationale
Appealed 2019 crosswalk recommendation. For 2020, requesting Gapfill for component codes plus additional procedures for non-GSP molecular analysis for comparable resources and work for special variant of unknown significance analytics	Test includes DNA Full Gene Sequencing and deletion/duplication. PLUS additional procedures: MLPA for PMS2, aCGH for del/dup and Sanger for low quality reads as indicated

PLA Code 0129U

Hereditary breast cancer–related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis and deletion/duplication analysis panel (ATM, BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, and TP53)

	Full Text
Appealed 2019 crosswalk recommendation. For 2020, requesting Gapfill for component codes plus additional procedures for non-GSP molecular analysis for comparable resources and work for special variant of unknown significance analytics	Test includes DNA Full Gene Sequencing and deletion/duplication. PLUS additional procedures: MLPA or aCGH for del/dup and Sanger for low quality reads as indicated

PLA Code Set 2 (+1057U- +0162U)

Code		CX
+0157U	APC +RNAInsight	81201/NLA
+0158U	MLH1 +RNAInsight	81292/NLA
+0159U	MSH2 +RNA Insight	81295/NLA
+0160U	MSH6 +RNA Insight	81298/NLA
+0161U	PMS2 +RNA Insight	81317/NLA
+0162U	Lynch Panel (4 genes) +RNA Insight	81435/NLA

PLA Code Set 2 Codes 0157U-0162U

Code		Full Text
+0157U	APC +RNAInsight	APC (APC regulator of WNT signaling pathway) (eg, familial adenomatosis polyposis [FAP]). **RNA analysis to screen for abnormal RNA transcripts and to resolve DNA variants of unknown significance when indicated [1 gene].**
+0158U	MLH1 +RNAInsight	MLH1 (mutL homolog 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome). **RNA analysis to screen for abnormal RNA transcripts and to resolve DNA variants of unknown significance when indicated [1 gene].**
+0159U	MSH2 +RNA Insight	MSH2 (mutS homolog 2) (eg, hereditary colon cancer, Lynch syndrome) **RNA analysis to screen for abnormal RNA transcripts and to resolve DNA variants of unknown significance when indicated [1 gene].**
+0160U	MSH6 +RNA Insight	MSH6 (mutS homolog 6) (eg, hereditary colon cancer, Lynch syndrome). **RNA analysis to screen for abnormal RNA transcripts and to resolve DNA variants of unknown significance when indicated [1 gene).**
+0161U	PMS2 +RNA Insight	PMS2 (PMS1 homolog 2, mismatch repair system component) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome). **RNA analysis to screen for abnormal RNA transcripts and to resolve DNA variants of unknown significance when indicated [1 gene].**
+0162U	Lynch Panel (4 genes) +RNA Insight	Hereditary colon cancer disorders (eg, Lynch syndrome) **RNA analysis to screen for abnormal RNA transcripts and to resolve DNA variants of unknown significance when indicated [4 genes].**

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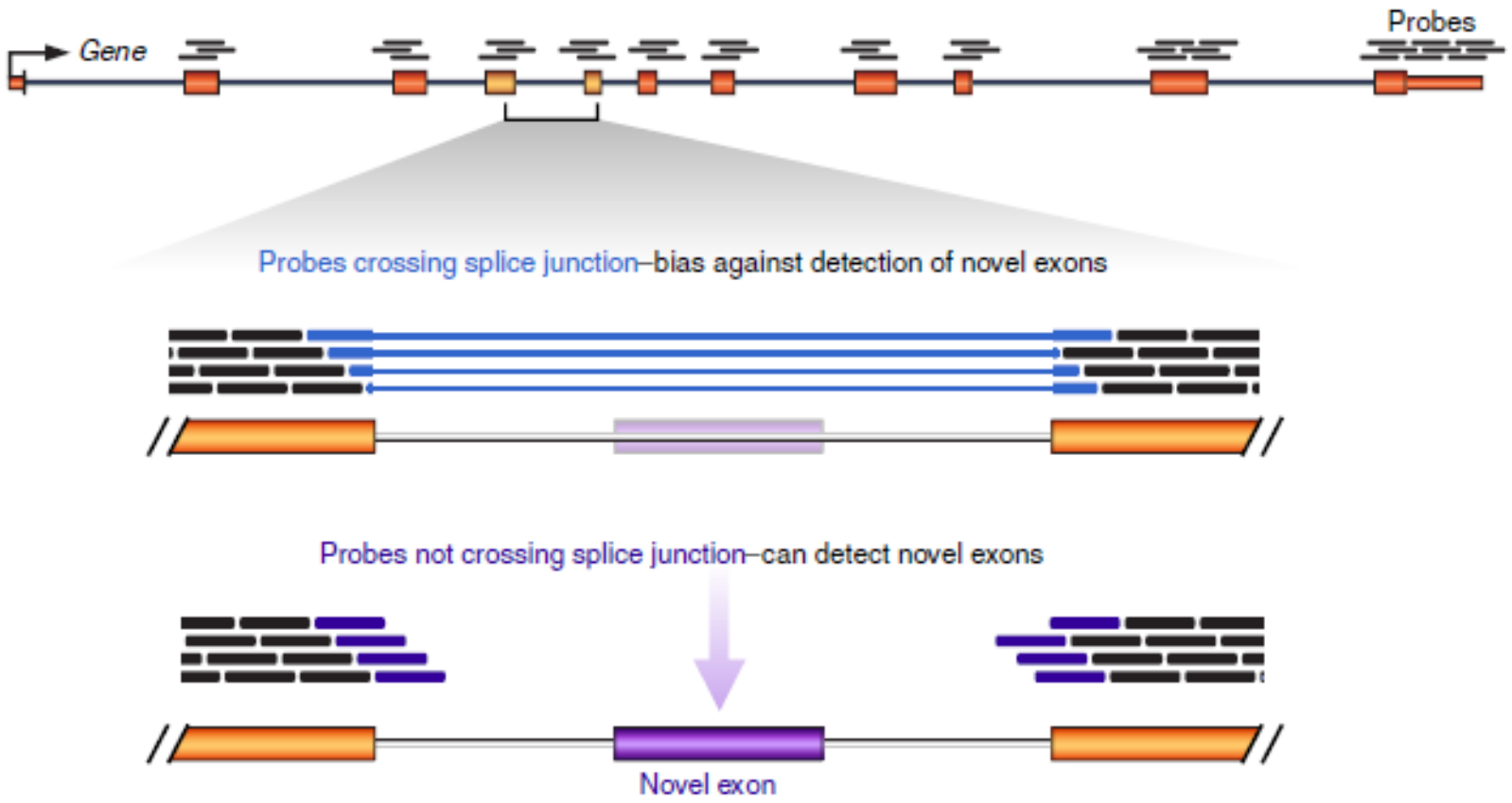
Effectiveness of Ambry mRNA analysis for Detection of Novel Variants (Intronic)

- Graphic & is by mRNA

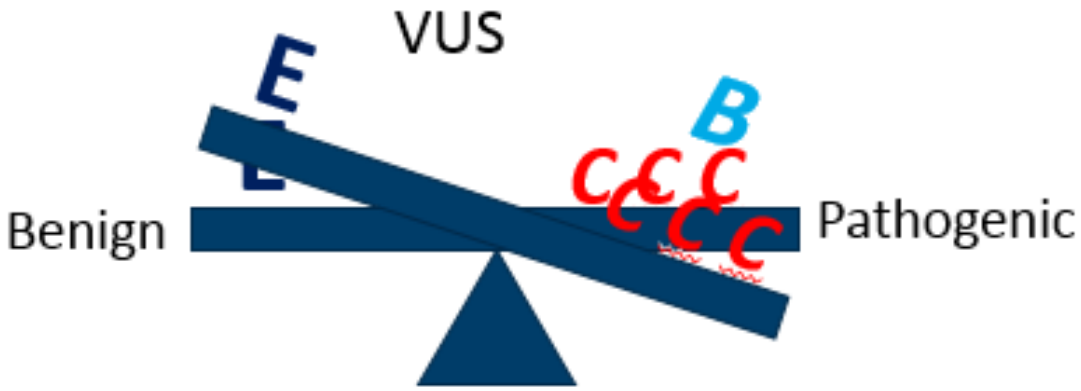


PLA Code Set 0101U-0103U

Effectiveness of Ambry mRNA analysis for VUS

Ambry's Multi-Evidence Method

- A – Pathogenic, Stand-Alone Evidence
  Functional Evidence (RNA)
- B – Pathogenic, Strong Level Evidence
- C – Pathogenic, Supporting Level Evidence
- D – Likely Benign, Supporting Evidence
- E – Likely Benign, Strong Level Evidence
- F –Benign, Stand-Alone Evidence



Richards S. *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24

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CMS Template Instructions (Online PDF)

https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Payment/ClinicalLabFeeSched/Downloads/CLFS-Annual-Laboratory-Meeting-Presentation-Template.pdf

Centers for Medicare and Medicaid Services (CMS)
Clinical Laboratory Fee Schedule (CLFS) Annual Laboratory Public Meeting

Instructions for completing presenter PowerPoint slide:

1. TITLE OF SLIDE: At the top of the PowerPoint slide insert the following:
   - Code Number (Text font and size: Calibri; 28)
   - Code Long Descriptor (Text font and size: Calibri; 28)
2. TABLE OF PUBLIC COMMENT + RATIONALE: Insert a table with the following column titles (table font and size; Calibri; 24):
   - Public Comment
     - In this column add the crosswalk code # and the NLA for that year in the cell under Public Comment header
   - Rationale
     - Add a brief rationale for the crosswalk in the cell under Rationale header

Public Comment	Rationale
Crosswalk code #, National Limit Amount (NLA) for that year	Provide brief rationale for crosswalk

3. SLIDE FOOTER: Add the name of your organization and name of the presenter (last name, first name) in slide footer. PLEASE DO NOT ADD Society or Company Logos.
4. For additional codes, insert an **additional slide** and repeat above steps

EXAMPLE of a completed PowerPoint slide:

80140: Calcitonin stimulation panel (eg, calcium, pentagastrin) This panel must include the following: Calcitonin (82308 x 3)

Public Comment	Rationale
82308 x3 ($20.00)	Consistent with code descriptor.

CMS Annual Lab Meeting, Date (MONTH DAY, YEAR)
Name of organization, Name of presenter (Last name, First name)

Important Notes:

- If either crosswalk code or gapfill is not recommended, please indicate as "N/A" in the rationale column
- Please use one slide per code.
- Please add to the footer the name of your organization or society.
- Please include the name of the speaker which will be needed to identify you during the webcast.
- Please refrain from using company logos.
- Remove any review comments
- Keep the background white
- Avoid using flickering/flashing text