Recommendations/Requirements for Molecular Proficiency Testing

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Legend of Terms

- CLIA = Clinical Laboratory Improvement Amendments
- EBV = Epstein-Barr virus
- FISH = Fluorescence in situ hybridization
- FFPE = Formalin-fixed, paraffin-embedded
- GIST = Gastrointestinal stromal tumor
- H&E = Hematoxylin and eosin stain
- HPV = Human papillomavirus
- ISH = In situ hybridization
- NGS = Next-generation sequencing
- PET = Paraffin-embedded tissue
- PT = Proficiency testing
- SNV = Single nucleotide variant
Additional Information Regarding CAP Survey Programs

• For additional information regarding the PT programs mentioned throughout these flow charts, please refer to the Surveys Catalog by clicking on the Catalog and Ordering Information link under the Laboratory Improvement header at [www.cap.org](http://www.cap.org).
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PT Requirements for Laboratories Accredited by the CAP

- Participation in PT is integral to the CAP’s accreditation program and is required for most tests for which the laboratory reports results.

- For analytes that require PT, each laboratory must enroll and participate in a CAP-accepted PT program. In the following flow charts, required programs/analytes will be indicated by an asterisk (*).

- For tests that do not require enrollment in a CAP-accepted PT program, the laboratory must perform an alternative assessment semi-annually to determine the reliability of testing. The most common way to do this is by purchasing an external PT product, if available. Other acceptable alternative assessment procedures are split sample analysis with reference or other laboratories, split samples with an established in-house method, assayed materials or other suitable and documented means. It is the responsibility of the director to define such alternative assessment procedures and the criteria for successful performance. Any program without an asterisk (*) in the following flow charts is not a required PT program and may be used to satisfy alternative assessment requirements. **Note:** International laboratories are required to enroll in CAP PT for all tests/activities if a CAP PT program is available.

- For a full list of required programs/analyte(s), please refer to the Analyte/Procedure Index in the Surveys Catalog.

- **Note:** the paths within the following flowcharts are not mutually exclusive.
• The NGS—Solid Tumor (NGSST), NGS—Hematologic Malignancies (NGSHM), and NGS—Germline (NGS) programs can be used to fulfill alternative assessment requirements for laboratories performing both wet bench and bioinformatic components of the assay. If a distributive testing model is used (eg, different parts of the NGS assay are performed by laboratories with different CLIA numbers), laboratories cannot use these programs for alternative assessment. To do so, laboratories would be subject to sanctions for PT referral.

• Laboratories using any other distributive testing process must use alternative approaches to fulfill the requirement for alternative assessment. Please note that distributive testing laboratories can use PT materials for part of their laboratory quality management program; laboratories should contact the CAP for additional details.
Are your laboratory's activities gene-specific (eg, cystic fibrosis CFTR screening)?

Does your laboratory do NGS panels, which include reporting/interpreting of specific genes (eg, CFTR associated mutations as part of a large NGS carrier panel)? ▲

Does your laboratory do Sanger sequencing on specific genes (eg, cystic fibrosis CFTR screening)?

Does your laboratory do whole genome or exome sequencing via NGS?

Is the analyte(s) required by the CAP Laboratory Accreditation Program?

Then your laboratory must enroll in gene-specific PT such as the MGL2* or MGL5* Survey

Then your laboratory may enroll in gene-specific PT (such as ICSP or CMSP) to satisfy alternative assessment requirements. Otherwise, an alternative assessment scheme, approved by the laboratory director, must be performed

If gene-specific PT is not available or not required, your laboratory may enroll in a methods-based Sanger sequencing (SEC or SEC1) program to satisfy alternative assessment requirements

Your laboratory may enroll in the methods-based NGS-Germline program or NGSE for undiagnosed disorders to satisfy alternative assessment requirements

Additional gene-specific PT programs:
AAT*, APOE*, BRCA*, CMSP, HGM*, ICSP, IMD*, MGL1-5*, PGX*, RETT*, and TPM*

*CAP Accreditation Program required program/analyte. Any program without an asterisk (*) reflected in this flow chart is not a required PT program; refer to page 4 for information regarding alternative assessment.

▲ A panel is defined as the reporting/interpreting of specific genes on a consistent, ongoing basis, regardless of technical approach (eg, performing exome sequencing on a preselect group of genes would be considered a panel). NOTE: A panel may contain required and non-required analytes; laboratories must enroll in gene-specific PT for required analytes and may use current PT programs to satisfy alternative assessment requirements for non-required analytes.
Germline Molecular FAQs

Q: My laboratory performs a hearing loss panel by NGS in which we report findings for 100 genes, including \textit{GJB2} (Connexin 26). Which PT program should I enroll in?
A: Your laboratory must enroll in gene-specific PT for Connexin 26 (MGL3* Survey) if it is accredited by the CAP. If there is no gene-specific PT for the remaining genes, your laboratory may enroll in the NGS-Germline program to satisfy alternative assessment requirements. Participation in MGL3* for Connexin 26 (\textit{GJB2} gene) will not satisfy alternative assessment requirements for the entire hearing loss panel.

Q: My laboratory tests for rare disorders (eg, Aarskog-Scott syndrome, Von Hippel-Lindau syndrome) by sequencing. What CAP PT is available to satisfy alternative assessment requirements for this assay?
A: Since there is not gene-specific PT available, your laboratory can enroll in the SEC or SEC1 Survey to satisfy alternative assessment requirements for Sanger sequencing and the NGS-Germline Survey to satisfy alternative assessment requirements for NGS. All three Surveys are considered methods-based programs.

Q: My laboratory does exome sequencing on diagnostic odyssey specimens. We report pathogenic/likely pathogenic and variants of uncertain significance that are present in any gene that fits the phenotype. What CAP PT is available to satisfy alternative assessment requirements for this assay?
A: In a case like this, laboratories may enroll in the NGSE program to satisfy alternative assessment requirements.

*CAP Accreditation Program required program/analyte.
Start

Does your laboratory perform molecular testing on PET?

No

Does your laboratory perform molecular testing on hematologic malignancies?

No

Does your laboratory perform molecular testing on solid tumors?

No

Stop

Yes

Refer to Hematologic Malignancy Flow Chart

Yes

Refer to Solid Tumor Flow Chart

Yes

Order MHOS

Yes

Order NEO

Yes

Stop

The testing reflected in this flow chart requires alternative performance assessment; the PT programs listed are not required. Refer to page 4 for information regarding alternative assessment.
Does your laboratory test gliomas? No: Order MSI
Yes: Order GU

Does your laboratory test for sarcoma translocations? No: Does your laboratory test for GIST associated mutations by non-NGS methods? No: Order RNA
Yes: Order SARC

Does your laboratory use RNA sequencing to detect solid tumor gene fusions? No: Does your laboratory perform cell-free, circulating tumor DNA analysis on plasma? No: Does your laboratory test for additional bioinformatic challenges? No: Does your laboratory want challenges in microsatellite instability? No: Does your laboratory want challenges in microsatellite instability? Yes: Order MTP

Does your laboratory use the Illumina TruSeq Amplicon Cancer panel? No: Does your laboratory use the Ion Torrent AmpliSeq Cancer Hotspot v2 panel? No: Does your laboratory use RNA sequencing to detect solid tumor gene fusions? Yes: Order NGSST

Does your laboratory test for genes other than BRAF, KRAS, and EGFR using NGS? No: Does your laboratory test for GIST associated mutations by non-NGS methods? No: Does your laboratory test for other DNA based mutations? Yes: Order individual gene Surveys (eg, BRAF, KRAS, and EGFR) OR MTP

NOTE: If there are alternative assays used to confirm NGS findings, these should also have appropriate alternative assessment performed. Alternative assessment options can include participation in CAP PT programs.

*BRAF, KRAS, and EGFR are CAP Accreditation Program required analytes. Laboratories must enroll in the MTP Survey or the individual gene programs (EGFR, KRAS and BRAF). Any program without an asterisk (*) reflected in this flow chart is not a required PT program; refer to page 4 for information regarding alternative assessment.
Does your laboratory perform DNA testing for IGH, IGK, TRB, or TRG rearrangements; or IGH/BCL2 or IGH/CCND1 translocations?

Yes: Order MHO or MHO1

No: Does your laboratory perform quantitative testing for BCR/ABL1?

Yes: Order MRD2

No: Does your laboratory perform qualitative and/or quantitative testing for BCR/ABL1?

Yes: p210: order MRD, p190: order MRD1

No: Does your laboratory perform testing for SNVs and small indels found in hematologic/lymphoid malignancies by NGS?

Yes: Order NGSHM

No: Does your laboratory perform sequence analysis of IGHV to determine SHM status?

Yes: Order IGHV

No: Does your laboratory perform qualitative testing for CEBFB/MYH11, RUNX1/RUNX1T1, PML/RARA, BCR/ABL1, CALR, MLL-PTD and other mutations (JAK2 V617F, FLT3 ITD and/or FLT3 TKD, NPM1)?

Yes: Order MHO2 or MHO3

No: Does your laboratory perform qualitative testing for CEBFB/MYH11, RUNX1/RUNX1T1, PML/RARA, BCR/ABL1, CALR, MLL-PTD and other mutations (JAK2 V617F, FLT3 ITD and/or FLT3 TKD, NPM1)?

Yes: Order MHO2 or MHO3

No: Does your laboratory perform qualitative testing for CEBFB/MYH11, RUNX1/RUNX1T1, PML/RARA, BCR/ABL1, CALR, MLL-PTD and other mutations (JAK2 V617F, FLT3 ITD and/or FLT3 TKD, NPM1)?

Yes: Order MHO2 or MHO3

Stop

*The testing reflected in this flow chart requires alternative performance assessment; the PT programs listed are not required. Refer to page 4 for information regarding alternative assessment.

Contains specimens in duplicate.

**NOTE:** If there are alternative assays used to confirm NGS findings, these should also have appropriate alternative assessment performed. Alternative assessment options can include participation in CAP PT programs.
Molecular Oncology FAQs

Q: My laboratory performs a 50 gene NGS-based assay designed to detect somatic SNVs and small indels observed in solid tumors. What CAP PT is available to satisfy alternative assessment requirements for this assay?
A: *BRAF, KRAS, and EGFR* are CAP Accreditation Program required analytes and laboratories must enroll in the MTP* program or the individual gene programs (EGFR*, KRAS*, or BRAF*), regardless of the methodology used. The laboratory may enroll in the NGSST program to satisfy alternative assessment requirements for the remaining genes in their NGS-based assay.

Q: My laboratory performs a 50 gene NGS-based assay designed to detect somatic SNVs and small indels observed in solid tumors. In addition, we have individual Sanger sequencing-based assays for *KRAS, BRAF, and EGFR*. Can we use the NGSST program to satisfy alternative assessment requirements for all these analytes/genes?
A: In this case, the laboratory may order the NGSST program for their NGS-based solid tumor assay to satisfy alternative assessment requirements and must order either MTP* or the individual gene programs (EGFR*, KRAS*, or BRAF*) for *KRAS, BRAF, and EGFR*.

Q: My laboratory performs a 50 gene NGS-based assay designed to detect somatic SNVs and small indels observed in hematologic malignancies. What CAP PT is available to satisfy alternative assessment requirements for this assay?
A: The laboratory may enroll in the NGSHM program. It is not necessary to also enroll in MHO Survey for this assay.

*CAP Accreditation Program required program/analyte.*
Molecular Oncology FAQs (continued)

Q: My laboratory performs a 50 gene NGS-based assay designed to detect somatic SNVs and small indels observed in hematologic malignancies. In addition, we have individual PCR-based assays for JAK2, FLT3, and NPM1. Can we use the NGSHM program to satisfy alternative assessment requirements for all these analytes/genes?
A: In this case, the laboratory may order the NGSHM program for their NGS-based hematologic malignancy assay and MHO2 or MHO3 for the individual PCR-based assays if your laboratory chooses to use the CAPs PT programs to satisfy alternative assessment requirements.

Q: Our laboratory performs NGS-based testing for the detection of somatic copy number variants and structural variants in solid tumors. What CAP PT is available to satisfy alternative assessment requirements for these assays?
A: Currently, there are no CAP programs for NGS-based detection of copy number variants and structural variants. An alternative assessment scheme, approved by the laboratory director, must be performed (Sample Exchange Registry, etc.).

Q: Our laboratory performs NGS-based testing and would like additional bioinformatic challenges in addition to wet-bench challenges. Is there a PT program available for this that may be used to satisfy alternative assessment requirements?
A: Yes, the laboratory may enroll in either NGSB1, if they are using the Illumina TruSeq Amplicon Cancer panel, or NGSB2, if they are using the Ion Torrent AmpliSeq Cancer Hotspot v2 panel. At this time, the CAP does not suggest that laboratories performing other panel-based tests use these programs. Additionally, there is a somatic validated materials (NGSBV) program available. This in silico program is designed to optimize bioinformatics pipelines, augment validations, and assist with pipeline verification after changes to NGS/bioinformatics processes. This is not traditional PT and no results will be returned to the CAP; information regarding the variants introduced will be sent along with the mutagenized file.
Start

Does your laboratory test FFPE samples?

- Yes
  - HER2 gene Amplification?
    - Yes
      - Constitutional/ neoplastic disorders?
        - Yes: Order CYF
        - No: Order CYH
    - No: Kappa/ Lambda, EBV, and/or HPV?
      - Yes
        - Glioma (1p/19q)?
          - Yes: Order CYJ
          - No: Solid Tumor?**
            - Yes: Order CYX
            - No: Lymphoma?
              - Yes: Order CYL
              - No: Brightfield

- No
  - Does your laboratory test non – FFPE samples (fixed cell suspensions*)?
    - Yes
      - Urothelial carcinoma?
        - Yes: Order CYI*
        - No: Stop
    - No: Order ISH

*CAP Accreditation Program required program/analyte. Any program without an asterisk (*) reflected in this flow chart is not a required PT program; refer to page 4 for information regarding alternative assessment.

*CAP Accreditation Program required analyte applies to kappa/lambda and EBV only.

**Challenges rotate between sarcomas, neuroblastomas, gastric carcinoma (HER2), and lung (ALK) cancer/carcinoma.

^ Other preparations (touch preparations, smears, etc.) – alternative assessment required.

ISH/FISH interpretation only – alternative assessment required (split sample exchange, etc.).
Does your laboratory do cytogenomic microarray analysis?

Yes

Constitutional testing?

Yes

Order CYCGH

No

Oncology testing?

Yes

Order CYCMA

No

Start

Does your laboratory do expression arrays?

No

Does your laboratory do expression arrays?

Yes

Order CYCMA

Stop

An alternative assessment scheme, approved by the laboratory director, must be performed

*CYCGH PT is not applicable to preimplantation genetic diagnosis (PGD) or exon-level array testing. For PGD, alternative assessment is required. For exon-level arrays, gene-specific duplication/deletion PT may be available (eg, DMD, MECP2) to fulfill alternative assessment requirements or laboratories must identify another form of alternative assessment.

* The testing reflected in this flow chart requires alternative performance assessment; the PT programs listed are not required. Refer to page 4 for information regarding alternative assessment.
Additional Information for Microbiology and Histocompatibility:

Microbiology:
• If performing patient testing on specimens by molecular methods only, laboratories must meet the regulatory requirements of testing five specimens in three mailings for each subspecialty, as appropriate. Subspecialties include Bacteriology, Mycology, Virology, and Parasitology. The Mycobacteriology requirement is five specimens tested in each of the two mailings.

• If performing molecular testing on patient specimens, in addition to traditional culture methods, alternative assessment is required. Alternative assessment can be met through enrollment in PT programs.

Histocompatibility:
• Regardless of methodology, laboratories should enroll in the appropriate HLA program(s) to meet testing needs.