Recommendations/Requirements for Molecular Proficiency Testing

Published Date:
9/12/2022
Legend of Terms

- CLIA = Clinical Laboratory Improvement Amendments
- CNV = Copy number variant
- 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
- SHM = Somatic hypermutation
- 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.
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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.
PT Referral

• The NGS programs [NGS—Germline (NGS), NGS—Solid Tumor (NGSST) and NGS—Hematologic Malignancies (NGSHM)] are for laboratories performing wet bench, bioinformatic, and interpretative 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/CAP numbers), laboratories cannot participate in the NGS, NGSST, and/or NGSHM PT programs as is (ie, without modifications to the overall process). 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 PT enrollment/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)?

Yes

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

Yes

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

No

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

No

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

Yes

Your laboratory may enroll in the methods-based NGS-Germline program

No

Stop

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

Yes

Stop

No

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

Yes

Does your laboratory want additional bioinformatic challenges?

Yes

Refer to the NGS Bioinformatics Flow Chart

No

No

Additional gene-specific PT programs:

AAT*, APOE*, BRCA*, CMSP, HGM*, ICSP, IMD*, MGL1-5*, PGX*, RETT*, and TPM*

▲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 nonrequired analytes; laboratories must enroll in gene-specific PT for required analytes and may use current PT programs to satisfy alternative assessment requirements for nonrequired analytes.

• 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.

*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 5 for information regarding alternative assessment.
Germline Molecular FAQs

Q: My laboratory performs a hearing loss panel by NGS in which we report findings for 100 genes, including **GJB2** (Connexin 26). Which PT program should I enroll in?
A: Your laboratory must enroll in gene-specific PT for Connexin 26 (MGL3* program) 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 (**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 no gene-specific PT available, your laboratory can enroll in the SEC or SEC1 program to satisfy alternative assessment requirements for Sanger sequencing and the NGS-Germline program to satisfy alternative assessment requirements for NGS. All 3 programs 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 (proband only) or NGSET program (proband plus parents) to satisfy alternative assessment requirements.

*CAP Accreditation Program required program/analyte.
*The testing reflected in this flow chart requires alternative performance assessment; the PT programs listed are not required. Refer to page 5 for information regarding alternative assessment.
*Note: If there are alternative assays used to confirm NGS findings, these should have appropriate alternative assessment performed. Alternative assessment options can include participation in CAP PT programs.
Hematologic Malignancy Flow Chart

Start

Does your laboratory perform DNA testing for lymphoma IGH, IGK, TRB, or TRG clonality or IGH::BCL2 or IGH::CCND1 translocations?

Yes

Order MHO* or MHO1**

No

Does your laboratory perform qualitative and/or quantitative testing for BCR::ABL1 for minimal residual disease?

Yes

p210: order MRD
p190: order MRD1

No

Does your laboratory perform quantitative testing for PML::RARA for minimal residual disease?

Yes

Order MRD2

No

Does your laboratory perform qualitative testing for myeloid translocation CEBFB::MYH11, RUNX1::RUNX1T1, PML::RARA, BCR::ABL1 and mutations CALR, KMT2A-PTD (MLL-PTD), MPL, JAK2 V617F, FLT3 ITD, FLT3 TKD, and NPM1?

Yes

Order MHO2* or MHO3**

No

Does your laboratory perform additional bioinformatic challenges?

Yes

Refer to the NGS Bioinformatics Flow Chart

No

Does your laboratory perform testing for SNVs and small indels found in hematologic/lymphoid malignancies by NGS?

Yes

Order NGSHM***

Stop

No

Does your laboratory perform sequence analysis of IGHV to determine rearrangement and SHM status?

Yes

Order IGHV

Stop

*CAP Accreditation Program required programs/analyte. Any program without an asterisk (*) reflected in this flow chart is not a required PT program; refer to page 5 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 PT program should I enroll in?
A: Enrollment in NGSST* is required for CAP-accredited laboratories.

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, KIT, BRAF, and EGFR. Can we use the NGSST program to satisfy requirements for all these analytes/genes?
A: In this case, the laboratory must order the NGSST* program for their NGS-based solid tumor assay. The laboratory may order either MTP* or the individual gene programs (KRAS*, KIT*, BRAF* or EGFR*) for the KRAS, KIT, BRAF, and EGFR Sanger sequencing assay, but is not required to.

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 program should I enroll in?
A: Enrollment in NGSHM* is required for CAP-accredited laboratories.

*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 requirements for all these analytes/genes?
A: In this case, the laboratory must order the NGSHM* program for their NGS-based solid tumor assay. The laboratory may order MHO2* or MHO3* for the individual PCR-based assays, but is not required to.

Q: Our laboratory performs NGS-based testing for the detection of somatic CNV and structural variants in solid tumors. What CAP PT is available to satisfy alternative assessment requirements for these assays?
A: In this case, the laboratory can enroll in the CNVST program for NGS solid tumor CNV analysis to satisfy alternative assessment requirements. Currently, there are no CAP programs for NGS-based detection of structural variants, therefore 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 (panel-specific) or NGSB4 for solid tumor challenges or NGSB3 (panel-specific) or NGSB5 for hematologic malignancy challenges. Additionally, there is a somatic validated materials portion available with the NGSB4 and NGSB5 programs, which are designed to optimize bioinformatics pipelines, augment validations, and assist with pipeline verification after changes to NGS/bioinformatics processes in addition to offering PT. Refer to the NGS Bioinformatics Flow Chart for additional information.

*CAP Accreditation Program required program/analyte.
Does your laboratory use any of the following panels?
- Illumina TruSeq Amplicon Cancer Panel
- Illumina TruSight Tumor 15 Panel
- Illumina TruSight Tumor 170 Panel
- Illumina TruSight Oncology 500 Panel
- Thermo Fisher Ion AmpliSeq Cancer Hotspot Panel v2
- Thermo Fisher Oncomine Comprehensive Assay v3
- Thermo Fisher Oncomine Focus Cancer Panel

Does your laboratory want additional bioinformatic challenges?

For Solid Tumor?

For Hematologic Malignancies?

For Germline Exome Analysis for Undiagnosed Disorders?

Stop

Yes

Order NGSB3

No

Order NGSB4

Yes

Order NGSB1

No

Order NGSB5

Yes

Proband only?

Yes

Order NGSE

No

Trio (proband + parents)?

Yes

Order NGSET

No

No

The testing and PT programs reflected in this flow chart are not required.
▲ These programs require laboratories to submit FASTQs or unaligned BAMs to CAP for in silico mutagenesis. In addition to the PT portion, NGSB4 and NGSB5 also contain a validated materials portion designed to optimize NGS/bioinformatics processes.
**Start**

**Does your laboratory test FFPE samples?**
- Yes
  - **ERBB2 (HER2) Amplification?**
    - Yes
      - Order CYI*
    - No
      - **Kappa/ Lambda, EBV, and/or HPY?**
        - Yes
          - Order ISH*
        - No
          - **Gloma (1p/19q)?**
            - Yes
              - Order ISH2*
            - No
              - **Solid Tumor?**
                - Yes
                  - Order CYJ
                - No
                  - **Lymphoma?**
                    - Yes
                      - Order CYK
                    - No
                      - **Lung Cancer?**
                        - Yes
                          - Order CYALK
                        - No

- No
  - **Does your laboratory test non FFPE samples (fixed cell suspensions)?**
    - Yes
      - Urothelial carcinoma?
        - Yes
          - Order CYI*
        - No
          - Constitutional/ neoplastic disorders?
            - Yes
              - Order CYF
            - No
              - **Brightfield**
                - Yes
                  - FISH
                    - Yes
                      - Order CYI*
                    - No
                      - Order CYH*
                - No
                  - **Order CYJ**
    - No
      - **Order CYL**

**Stop**

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*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 5 for information regarding alternative assessment.

**Challenges rotate between sarcomas, neuroblastomas, gastric carcinoma [ERBB2 (HER2)], and lung cancer/carcinoma.

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

ISH/FISH interpretation only – alternative assessment required (split sample exchange, etc.).
The testing reflected in this flow chart requires alternative performance assessment; the PT programs listed are not required. Refer to page 5 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 5 specimens in 3 mailings for each subspecialty, as appropriate. Subspecialties include bacteriology, mycology, virology, and parasitology. The mycobacteriology requirement is 5 specimens tested in each of the 2 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.