

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.

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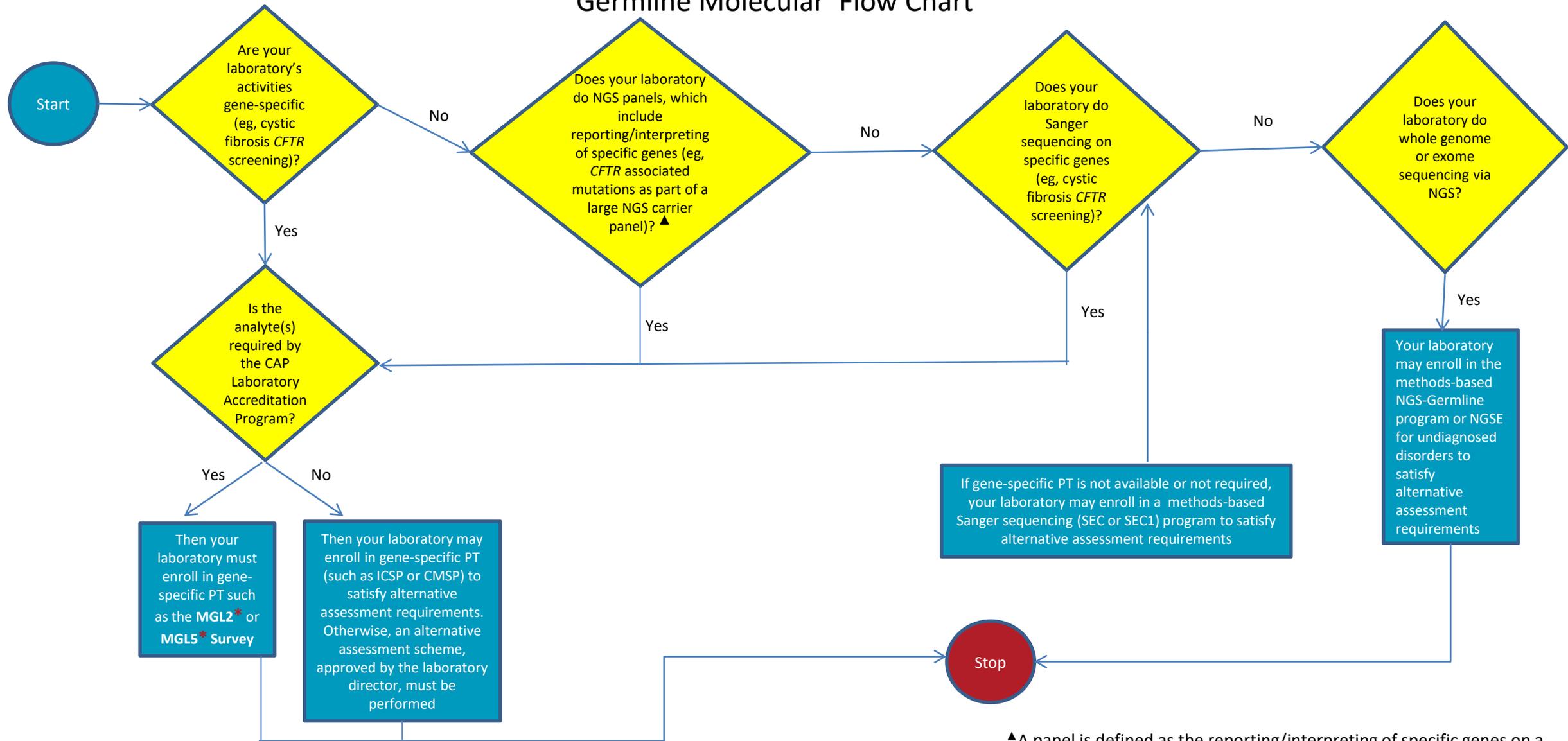
PT Requirements for Laboratories Accredited by the CAP

- 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—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/CAP 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.

Germline Molecular Flow Chart



▲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.

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.

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* 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 (*GJB2* gene) will not satisfy alternative assessment requirements for the entire hearing loss panel.

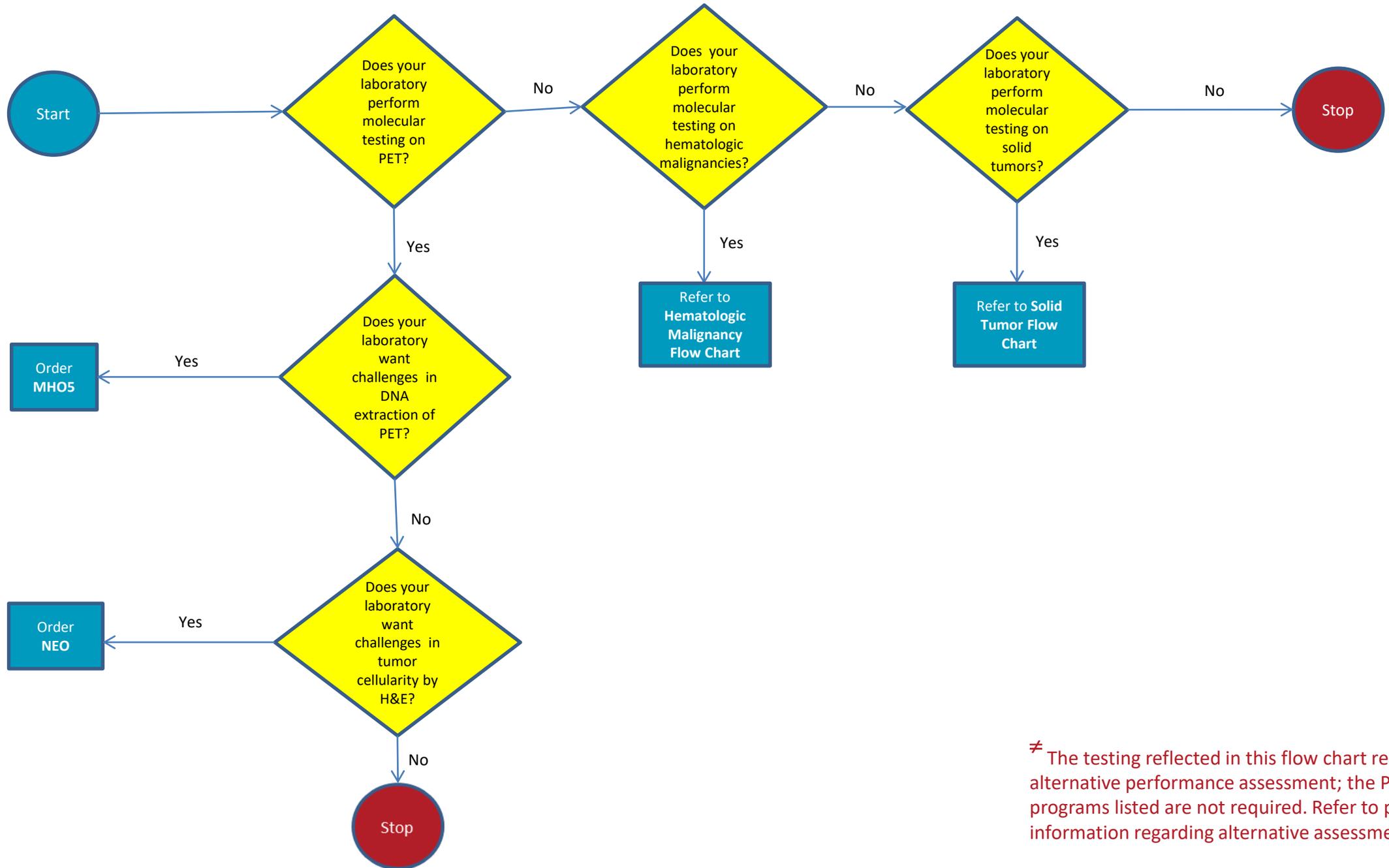
Q: My laboratory tests for rare disorders (eg, Aarskog-Scott syndrome, Von Hippel-Lindau syndrome) by sequencing. What CAP PT is available to satisfy alternative assessment requirements for this assay?

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?

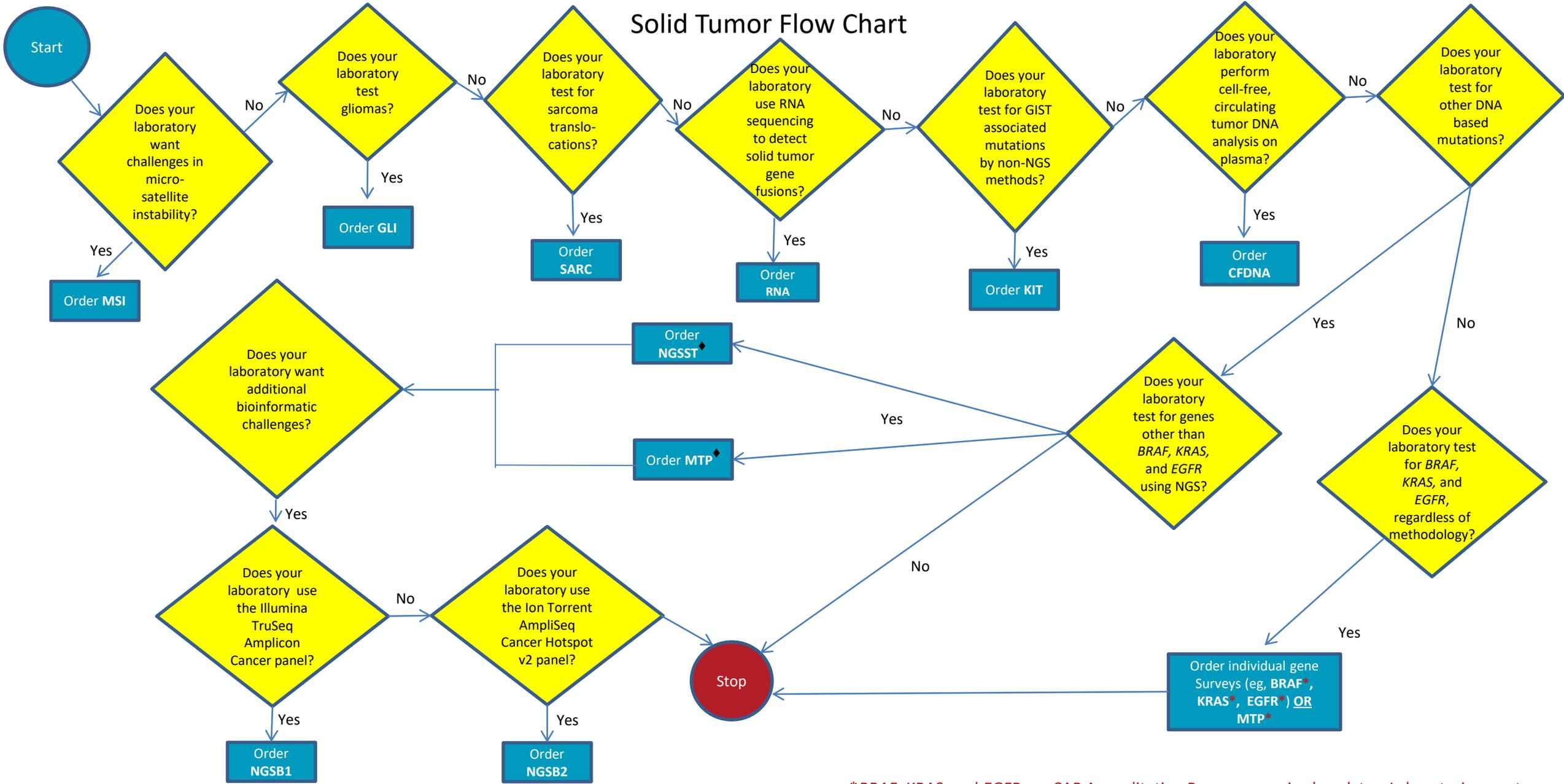
A: In a case like this, laboratories may enroll in the NGSE program to satisfy alternative assessment requirements.

General Molecular Oncology Flow Chart[≠]



[≠] 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.

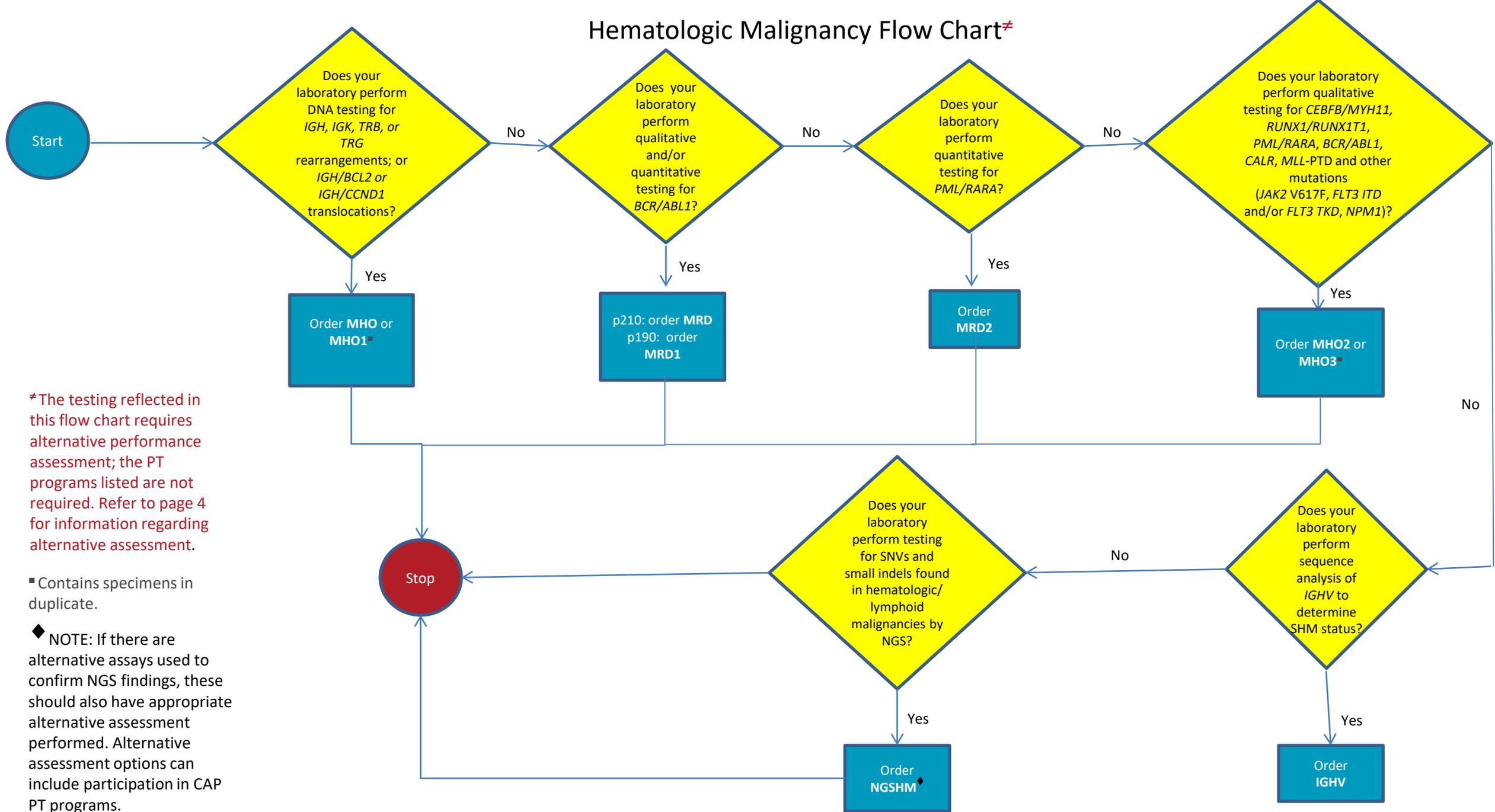
Solid Tumor Flow Chart



◆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.

Hematologic Malignancy Flow Chart[≠]



*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 NGSST 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.

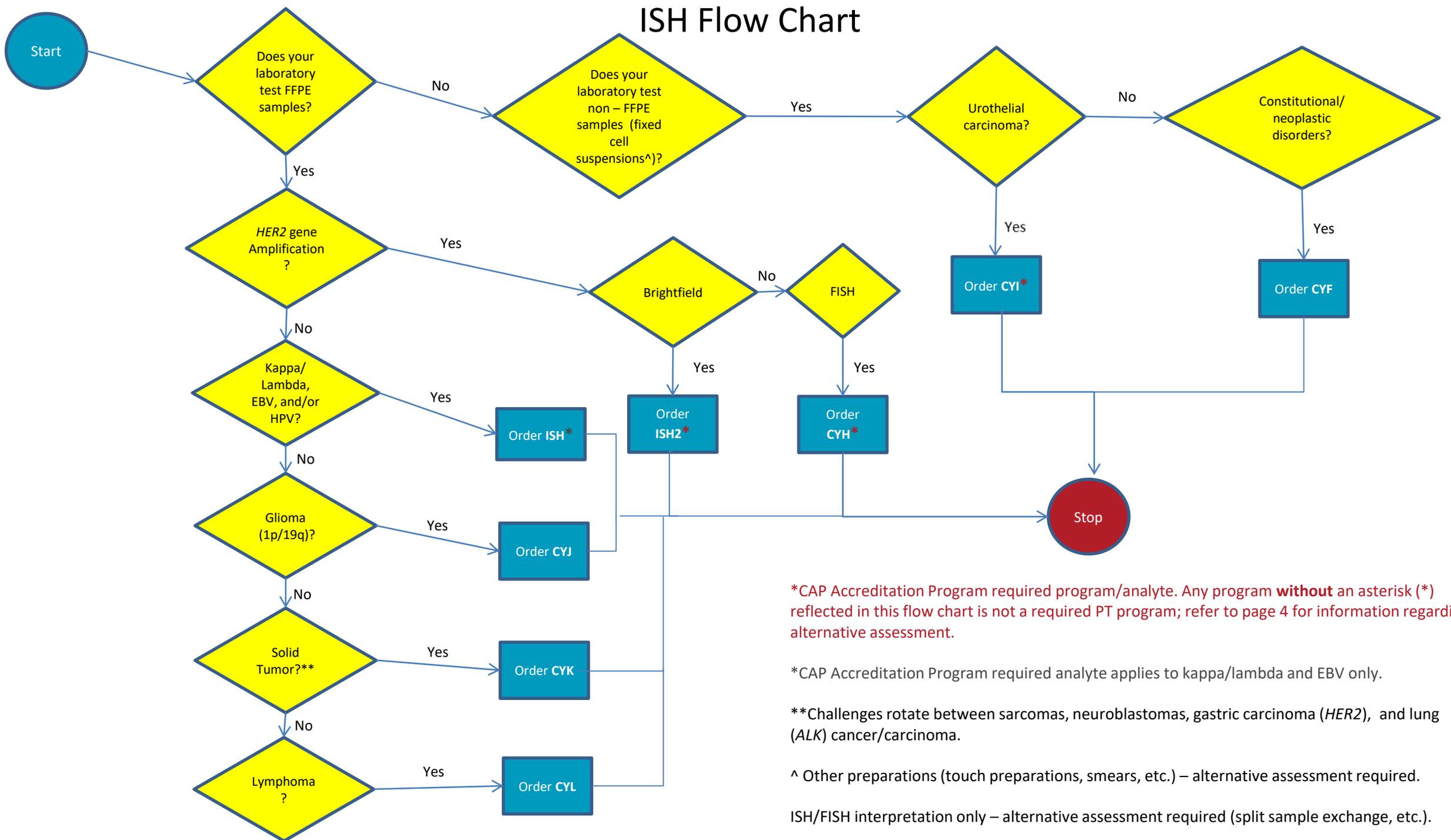
Q: Our laboratory performs NGS-based testing for the detection of somatic copy number variants and structural variants in solid tumors. What CAP PT is available to satisfy alternative assessment requirements for these assays?

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.

ISH Flow Chart



*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.

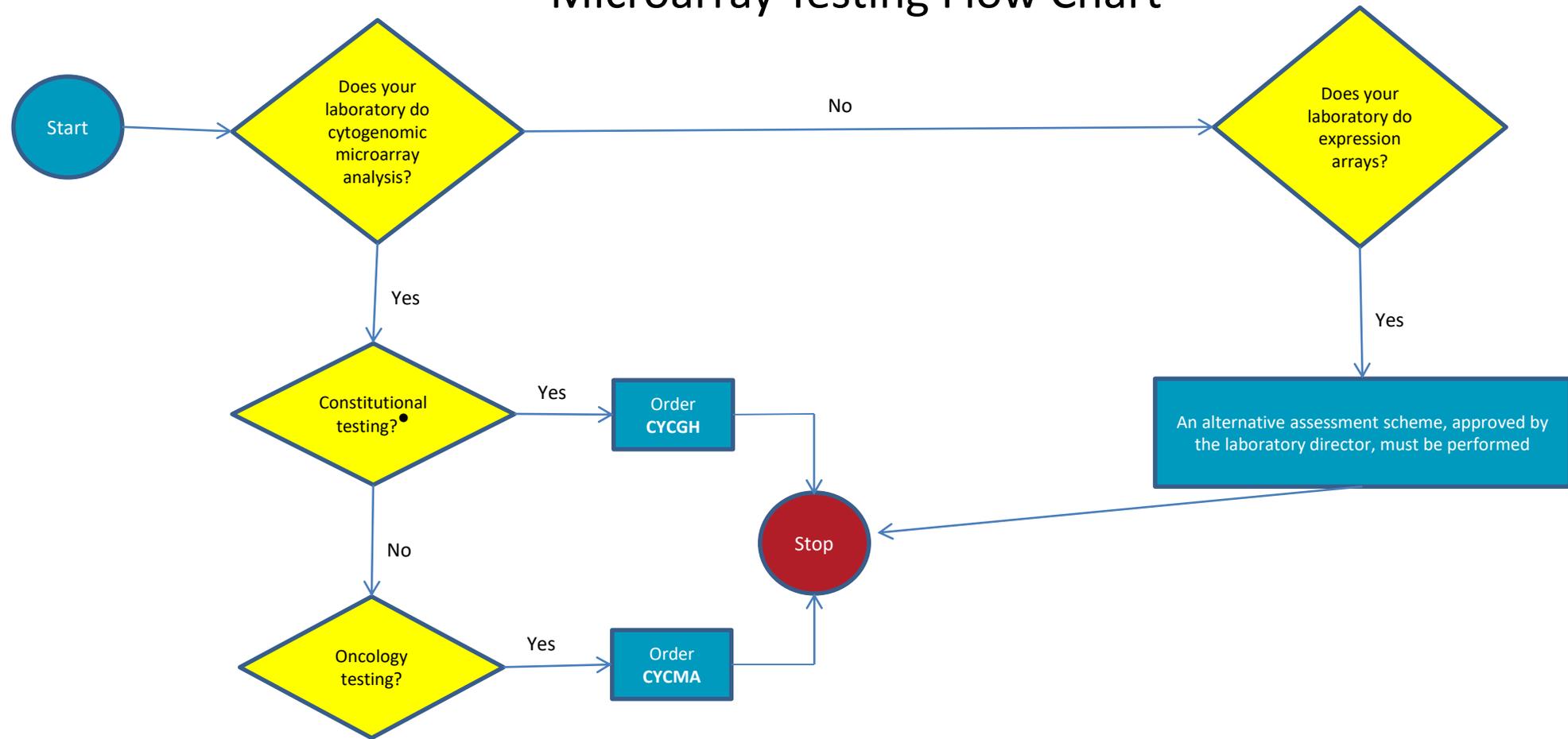
*CAP Accreditation Program required analyte applies to kappa/lambda and EBV only.

**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.).

Microarray Testing Flow Chart [≠]



[≠] 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.

•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.

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.



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