Penn Medicine

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To: UPHS Physicians and Staff

From: **The Division of Precision and Computational Diagnostics (PCD)**

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Re: **METHOD CHANGE: T-cell Receptor Gamma Gene Rearrangement Testing**

At the end of July, the Molecular Pathology Laboratory will be launching a new method for T-cell receptor gamma gene (TRG) rearrangement using next generation sequencing (NGS), LymphoTrack TRG Assay Panel-MiSeq (Invivoscribe, Inc.). The new sequencing method overcomes some of the disadvantages of the current capillary electrophoresis (CE) method for assessment of T cell clonality. For example, whereas interpretation of the CE results are somewhat subjective based on the ratio and pattern of peaks, interpretation of the new TRG clonality by NGS method is objective based on an algorithm which includes the frequency of specific V-J clonotype sequences and the fold over polyclonal background. Likewise, each individual clonotype sequence can be evaluated independent of the product size, unlike CE for which multiple rearrangements may have the same size and cannot be distinguished, leading to a high rate of indeterminate results. Accordingly, because the new NGS method is more sensitive and specific than CE, there will be fewer indeterminate results and a lower overall positivity rate with TRG clonality by NGS.

Similarities and differences between the two methods are summarized below:

Criteria	LymphoTrack Sequencing (New Method)	Capillary Electrophoresis (CE) (Old Method)
Method	PCR + paired-end NGS	PCR + capillary electrophoresis
Target region	Single PCR reaction with Vg 2-5 and 8-11 combined with J primer mix	Two PCR reactions with Vg 1-8 or Vg 9-11 primers combined with a J primer mix
Clinical sensitivity	No significant change	90-95% of T-cell lymphomas detected
Analytical sensitivity	At least 0.1%	1-10%
Comparison and low-level clonality	V-J genes, size, and sequence of clonotypes between samples and report low-level clonality	Limited specificity and sensitivity for comparison of clones between samples and tracking over time
Reporting	V-J gene usage, product size, and frequency (semi-quantitative)	Master mix and peak size
Acceptable samples	No change	Blood and bone marrow in Lavender tube Fresh tissue in Michel's media Formalin fixed paraffin-embedded tissue
Turn-around-time (TAT)	No change	7-10 business days

Additional Information: Test ordering is unchanged. Consult the Lab Tests Services Guide for ordering information and specimen requirements. If both IGH gene rearrangement and TRG gene rearrangement are ordered simultaneously, they will be reported separately instead of in a combined report. To request comparison of clonality to a sample previously tested by CE, add a comment on the manual requisition or in SPHEME or PTOE order. Testing of prior samples during the transition period is dependent upon residual material being available and may prolong the TAT.

To contact Molecular Pathology Laboratory, call 215-615-3094 weekdays during regular business hours.