CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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Enhancements to Tissue FISH (HER2/ALK) testing: Tissue Target Matching Application

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Effective Date: August 6, 2018

Testing Schedule: See testing schedule for each individual assay.

Specimen Types: No change. See Technical Bulletins No. 115a (HER2 FISH) and No. 118 ALK FISH) for details.

Clinical Use:

CPAL's FISH microscope platform has undergone significant upgrades, allowing the capability to process both bright field and fluorescent high-resolution images. The updates to the system allow the addition of the Tissue Target Matching Application to CPAL's existing FISH testing platform. System upgrades and software application additions will offer direct correlation of FISH targets to the indicated invasive areas on H&E/IHC slides.

H&E/IHC-stained slides for HER2 and ALK FISH testing are scanned using the bright-field camera on the Duet microscope platform. Invasive areas indicated by the submitting pathologist are electronically transcribed on the scanned H&E/IHC image. Electronic refinement of invasive areas will also now be possible. After sample processing for FISH target analysis has occurred, a separate slide is scanned using the fluorescence filter. The two images (bright field and fluorescence) are digitally overlaid by the Tissue Matching Application software. Two matching landmarks spanning the tissue section to be investigated are identified by the technologist performing FISH target image capture. The exact same landmarks are selected on both the bright field slide image and the fluorescent slide image. The Tissue Matching Software then fully-integrates the separate slide images for FISH target capture and analysis. The invasive areas indicated by the pathologist on the H&E/IHC slide are electronically transferred to the fluorescent

FISH slide. During review in SoloWeb, the exact locations of the fields of view selected for FISH target analysis on both the H&E and DAPI slide images, as well as the location of individual targets analyzed, are indicated to the ordering pathologist.

Validation Studies:

Previously analyzed slides were assessed for FISH image capture, tissue target matching, and target analysis from March 23, 2017 and March 27, 2017. FISH signal enumeration occurred from March 23 to March 28, 2017.

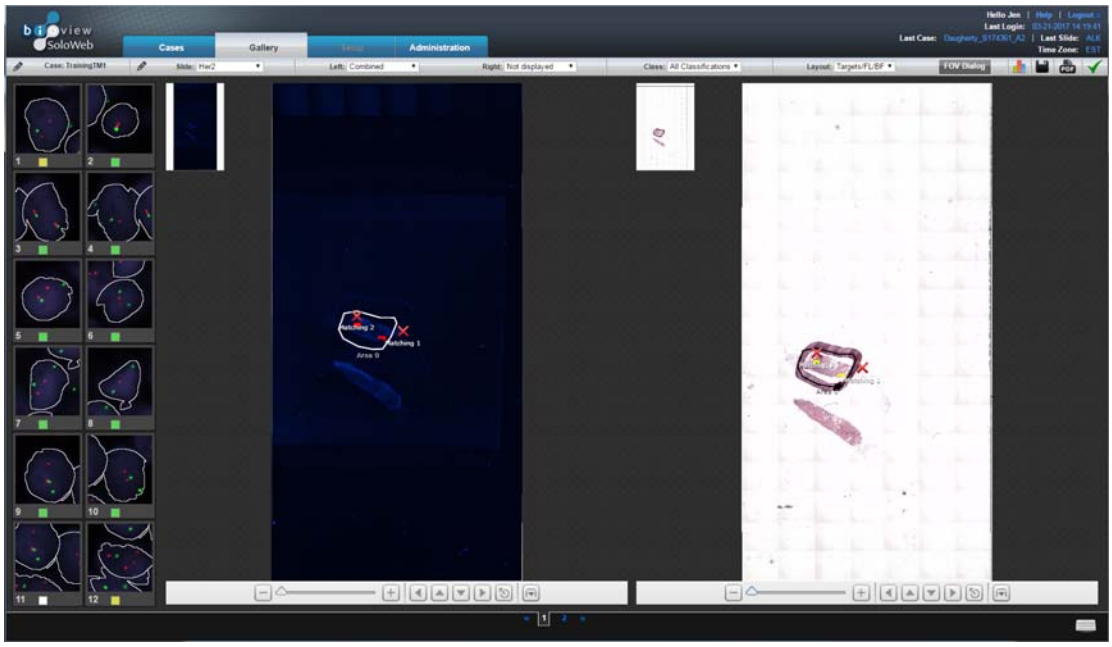
FISH signal quality and enumeration Data Comparison, Tissue Matching Application:

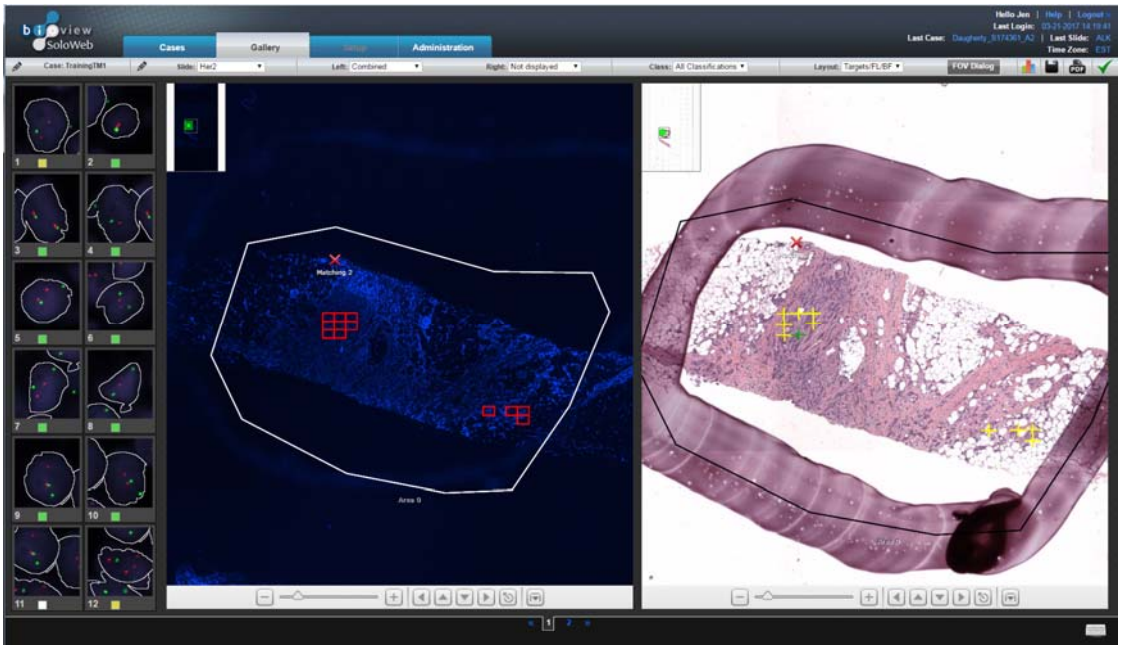
Validation ID	Prev. Result	New Results	Valid? (Y/N)
032317_Sample 1_ALK	Negative, 2%	Negative, 4%	Y
032317_Sample 2_ALK	Positive, 23%	Positive, 22%	Y
032817_Sample 3_ALK	Positive, 90%	Positive, 94%	Y
032417_Sample 1_HER2	Negative, Ratio= 1.10	Negative, Ratio= 1.06	Y
032317_Sample 2_HER2	Equivocal, Ave. HER2 copies/cell= 4.29	Equivocal, Ave. HER2 copies/cell= 4.20	Y
032417_Sample 3_HER2	Positive, Ave. HER2 copies/cell= 6.31	Positive, Ave. HER2 copies/cell= 6.20	Y

During the Tissue Matching Application validation, significant information technology (IT) shortcomings were identified. Additional upgrades were made to CPAL's networking capabilities over the course of several months and completed in January 2018. Upon completion of IT upgrades, an additional vendor software upgrade was required. This upgrade was completed in April of 2018. All IT/software updates were also validated. Data available upon request.

Discussion:

The Pathvysion HER2-*neu* and Vysis ALK FISH data were unaffected by the addition of the BioView Tissue Target Matching Application. All tissue section overlays were completed successfully; a HER2-neu example is depicted below. Based upon the results of these comparisons and verification of successful image overlay, clinical use of the Tissue Target Matching Application was approved for use.





Additional instructions and/or training will be provided to end-user pathologists who will use the upgrades to the SoloWeb software.

References:

1. Bioview Duet™ User's Manual, Version 10.0, June 2014, Rehovot, Israel
2. Bioview SoloWeb™ User's Manual, Version 2.0, June 2014, Rehovot, Israel
3. Bioview Addendum– Duet/Solo User's Manual, Version 2.0, September 2015, Rehovot, Israel