Utilization of Innovative Web Based FISH Review and Analysis Solution in Daily Clinical Practice

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Agenda

- Clinical laboratory, a challenging environment
  - Routine, technical, Scientific
- Addressing the lab challenges using the web based system
- Telepathology
- Addressing Telepathology requirements
- Overview of Bioview Implementation in our Lab
Clinical Laboratory, a Challenging Environment
Routine Challenges

- TAT major challenge in medium sized laboratories
- Resources for a medium size laboratory
  - Between different lab sites
  - Availability of pathologist
- Time consuming analysis
Solutions to Routine Challenges Using Bioview

- TAT major challenge in medium sized laboratories
  - 50 slides loaded to scan during off hours
  - All cases ready for analysis as techs come in

- Resources for a medium size laboratory
  - Resources available across different sites
  - Scanned case allotted across sites
  - Availability of pathologist any time as the case is available for online review

- Time consuming analysis
  - FISH for cervical cancer needs 2,000 cells to be analyzed
  - Case study
FISH-based HPV-Associated Cancer Test, FHACT®

- 3q26 gain (red)
- Cen7 (aqua)
- 5p15 gain (green) and
- 20q13 gain (gold)
Impact of Number of Cells Scored on Correlation of Copy Number Changes with Lesion Severity

≥ 3 signals per cell = positive for gain, irrespective of pattern

Differences in distributions and trends in the number of cells with chromosomal abnormalities across disease categories were determined using Kruskal-Wallis tests and nptrend, respectively.

At least 1,000 cells should be scored
## Overview of Cervical Cancer FISH Analysis

### Manual Analysis
- 2000 cells scored
- 4 loci scored
- 2 techs needed
- 1.5-2hrs per case
- Low level gains additional cells reviewed or the entire slide reviewed
- Case analysis limited to the number of microscopes

### Automated Analysis
- 2000 analyzed cells scored
- 4 loci present in the same view
- Cells categorized based on signal patterns
- 1 tech needed
- 30 mins per case
- Additional non analyzed cells are reviewed as a part of regular analysis
- Case analysis limited to the number of review stations or unlimited with a terminal server
Case Study 1
Cervical Cancer FISH (Normal)

4 color FISH with all loci in single view
Case Study 1
Cervical Cancer FISH

Scan and analysis time
Case Study 2
Cervical Cancer FISH (Abnormal)

4 color FISH sorted with multiple gains
Case Study 2
Cervical Cancer FISH

Scan and analysis time
In Summary Bioview Brings Solutions to time consuming analysis

- Reduction in scan time
- Sort cells based on gains/quality/size of the cells
- Viewing all the four loci on the same plane
- Ease to view multiple loci in a single view increasing sensitivity for low level multiple gain
- Ease to eliminate Clusters/overlapping cells from analysis
  - DAPI image to identify overlapping cells
Clinical Laboratory, a Challenging Environment

Technical Challenges

- Improve sensitivity to improve patient outcome
- Background noise vs the signal strength
- Choosing the right regions for FFPE FISH
  - Reviewing the images corresponding to a region of interest
Solutions for Technical Challenges using Bioview
H&E scan and identifying the regions of interest
Solutions for Technical Challenges using Bioview H&E and DAPI Scan (Tissue Matching)
Solutions for Technical Challenges using Bioview
Review of FISH Images

https://cyto.expanddx.com:8443/BioWeb/Classify.jsp?0.9807374253970109
New Technical Challenges with the Use of Bioview and Their Solutions

- Background signals vs actual signals
  - Ability to view and scroll each fluorophores under different planes
- Variation in the signal intensities
- Choosing cells from the representative regions
  - Possibility to review the cell distribution with signal patterns
Clinical Laboratory, a Challenging Environment
Scientific Challenges

- Equivocal cases in HER2 FISH
  - True Equivocal
  - Equivocal due to cherry picking cells
    - Possibility to review multiple FOVs
- Genetic heterogeneity in HER2
  - Possibility to review H&E again during FISH analysis
  - Matching H&E FOVs with the FISH FOVs
Scientific Challenges Solutions Using Web Based FISH Capture and Analysis System

- Equivocal cases
  - True Equivocal vs false positives
    - Possibility to review multiple FOVs
    - Choosing the representative cells
- Genetic heterogeneity
  - Possibility to review H&E again after FISH analysis
  - Matching H&E FOVs with the FISH FOVs
  - Web-based H&E review with identification of the area of interest
  - Case study
Case Study 3

- Case is IHC 2+ equivocal
- FISH results are non-amplified (1.4 ratio and 3.5 avg HER2 copy number)
- The 20 cells circled are taken from FOV 1-4
- FOV 5 is also from within the inscribed area on the slide (and many other FOVs), but all cells here are 2G2R and don't exemplify the IHC 2+ results
Case Study 3

- FOV 5 is also from within the inscribed area on the slide (and many other FOVs), but all cells here are 2G2R and don't exemplify the IHC 2+ results

- FOV5 vs the Case Analysis Based on FOV1-4
Scientific Challenges
Genetic Heterogeneity for HER2

- Intra tumoral heterogeneity
- Sub clonal diversity
- HER2 – 5% - 30%
- Increases subjectivity in HER2 interpretation by pathologist
Case Study 4

- IHC for her2neu called as 2+ and FISH
- It showed scattered positive cells (3+)
- Tumor region marked
- Review of the cells corresponding to IHC or H&E slide
- Cells positive for HER2 by IHC – amplified by FISH
Case Study 4
IHC, H&E and DAPI Image – Corresponding Cells with FISH Signals

DAPI Low mag. scan

IHC Low mag. scan

FISH images corresponding to cells 3+ by IHC

DAPI Zoomed image  IHC Zoomed image
Addressing GH Using Web Based System

- Whole tumor region scanned
- Scanning performed under 20X
- Quick review of the low magnification scan
- Identifying the representative region in case of GH
- Pathologist can re-review the scan in case of discrepancy with IHC or in challenging cases
In Summary Bioview’s Web Based FISH Review and Analysis system brings solutions to Scientific Challenges

- Tissue matching between H&E and DAPI image is highly beneficial in equivocal cases and cases with genetic heterogeneity
- Reduction in review time
- Ease to view multiple loci in a single view
- Ease to eliminate Clusters/overlapping cells from analysis
Telepathology
Web based FISH

- Patient identification - Slide/Image ID
- Access to clinical information during analysis of the case
- Telepathology training
- Patient confidentiality
- Result documentation
- Quality management program
Addressing Telepathology Requirements

- **Patient identification - Slide/Image ID**
  - Bar coding

- **Access to clinical information during analysis of the case**
  - All the specimen information is present in the clinical history for reference

- **Telepathology training**
  - The technologists are trained and documents details of training is provided by Bioview who is assigned as champion
  - The champion trains all the pathologists and other techs who perform sign out and analysis respectively
Addressing Telepathology Requirements Contd.

- Patient confidentiality
  - System and user authentication is achieved by password protected user names for each remote pathologist
  - System provides the activity logs
  - Access restrictions is provided based on the user level
  - The images reside at the host server and users are given access via a password protected user name
- **Result documentation**
  - Diagnostics and specimen adequacy results are documented in the system
  - Report is generated consisting of the results of the review of the images/slides

- **Quality management program**
  - The laboratory is required to include QM plan
    - The system provides the statistics of TAT, inadequate, normal and abnormal cases
  - Comparison to onsite analysis can be performed by making copies of the case.
Innovative Web Based FISH Solution
Implementing Bioview in our lab

- Requirements and comparison with other systems
- High resolution images under low magnification
- Ability to handle large image files
- Provided by Bioview
  - Automated Microscope with 50 slide loader with a shock absorbance Equipment
  - In-house high capacity server
  - Review stations
Validation of the System

Validation of System for Internal Use

System validated against manual analysis

- Training the techs on usage of the system
- The number of cells to be captured
- The default signal patterns to be analyzed
- The review of all the signal patterns by the Tech
- Reporting of the case

Compliance

CAP requirements

- Slide /Image ID
- Clinical information access
- Telepathology training
- Telepathology and confidentiality (HIPAA)
- Telepathology result documentation
- Remote analysis and remote reporting validation
Conclusion

- Bioview brings solutions for some of the challenges in the Molecular cytogenetic laboratory
- CAP compliance, HIPAA requirements, 21 CFR Part II (Electronic Signature)
- Resources and staffing
- Scientific Challenges
- Market outreach
Thank you

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