Assessing and Qualifying Biospecimen Quality: Global Collaboration on Formalin-Fixed, Paraffin-Embedded Tissue Samples for the HIV/AIDS Cancer Related Research Biorepository.

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Abstract

Background: Archived formalin-fixed paraffin-embedded tissues (FFPET) are a resource for rapid translation from molecular target discovery to clinical disease application. NCI's AIDS Cancer and Specimen Resource (ACSR) provides HIV/AIDS-related malignancy tissue to approved investigators. Mid-Region ACSR has FFPET sampling techniques including field tissue microarrays (TMA) construction for cancer epidemiology or cancer molecular studies. TMAs facilitate high throughput immunohistochemistry (IHC) and in situ hybridization (ISH) evaluations and, with digital image analysis, allow cost-effective and time-effective evaluation.

Methods: Sampling and FFPET block production standards were used in US and East African ACSR sites for several years. FFPET quality at collaborator laboratories was assessed. Source FFPET cores were inserted into TMA blocks. TMA sections were stained and digitized. TMALab and ImageScope (Aperio) and *AnalyzeTMA* software facilitated image evaluation/sharing results. Quality of immunostaining, FISH, ISH, DNA and accuracy of pathology diagnosis/categorization were assessed.

Results: ACSR produced 8 TMA blocks (728 cores, 259 source blocks, various lymphomas). Cores for DNA isolation/characterization were also collected at the time of construction. RNA, DNA, IHC, and ISH were used for immunophenotyping and molecular genotyping. DNA isolated from samples was not of quality for molecular testing; signals from FISH probes were limited to few samples.

Conclusions: Digital imaging/software facilitated stained TMA tissue section evaluation. Resource-poor site samples were troubled by delayed fixation, poor quality formalin fixation, suboptimal tissue processing (particularly temperature control), poor slide preparation, and inconsistent quality pathology interpretation. Confusion of lymphoma with other small malignancy types and interpretation attempted from poor H&E stained slides occurred. Collaborative digital image review facilitates fixation/processing quality improvement at resource poor sites. Quality performance of FFPET for RNA, DNA, IHC, ISH and FISH excluded some tissues from the biorepository.

Background

Kenyatta National Hospital
Nairobi, Kenya

Mulago Hospital Complex
Kampala, Uganda

St Mary's Hospital Lacor

Histopathology Lab's first cassette

Gulu, Uganda

Figure 1: **(left to right) Nairobi, Kampala and Gulu sites**

Objectives

- Archived formalin-fixed paraffin-embedded tissues (FFPET) are a resource for rapid translation from molecular target discovery to clinical disease application. NCI's AIDS Cancer and Specimen Resource (ACSR) provides HIV/AIDS-related malignancy tissue samples and negative controls to approved investigators.
- The Mid-Region ACSR has developed FFPET sampling techniques including laboratory and field construction of tissue microarrays (TMA) from FFPET candidates for cancer epidemiology or cancer molecular studies. The TMAs facilitate the application of high throughput evaluations of immunohistochemistry (IHC), in situ hybridization (ISH) and allow cost/time-effective evaluation when coupled with digital imaging analysis.
- During clinical cancer treatment trials and cancer epidemiology studies, digital images are shared with the global collaborators to facilitate quality improvement protocols for tissue fixation and processing.

Methodology

- A standard for tissue sampling, fixation, processing and paraffin block production has been in use in ACSR operations in US and East African sites for several years.
- Collaborator laboratories have been assessed for tissue quality of collected/stored FFPET.
- Source FFPET cores were inserted into TMA blocks and TMA sections were produced, stained and digitally scanned.
- TMALab (Aperio), ImageScope (Aperio) and *AnalyzeTMA* software were used to facilitate image evaluation and to share results with collaborators.
- Quality of immunostaining, ISH, FISH, DNA and accuracy of pathology diagnosis or categorization were assessed.

Results

- The ACSR has produced 8 TMA blocks using 259 source blocks with a total of 728 0.6 and 1.0 mm cores from various lymphomas collected from global collaborators.
- Tissue cores for DNA isolation and characterization were also collected at the time of construction.
- RNA, DNA, IHC, and ISH were used to assess samples for immunophenotyping and molecular genotyping.
- DNA was isolated from samples but was not of quality for molecular testing and signals from FISH probes were limited to a few samples.

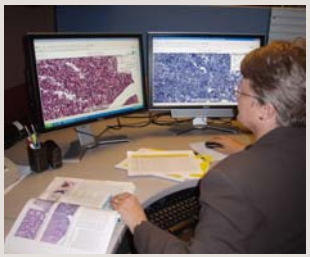

Figure 2: **(left) Barb Hackman assists Dr. Robert Lukonde (Uganda) in making a TMA; (right) Dr. Leona Ayers phenotypes lymphomas using digital images from TMAs.**

TMA ID	# cores	# donor blocks	core size (mm)	type of cores
TA06-001, 002	444	74	0.6	lymphoma QC study, HIV+ and HIV- (SFGH)
TA07-020	87	31	0.6	lymphoma, Kenya and Uganda
TA07-021	11	6	0.6	Burkitt Lymphoma, subset of TA07-020
TA07-022, 023	58	20	1	Burkitt lymphoma, Kampala, Uganda 1997 - 2006
TA07-030	74	74	1	Burkitt lymphoma, Gulu, Uganda 1993 - 2000
TA07-031	54	54	1	Burkitt lymphoma, Gulu, Uganda 2001 - 2005
total 8	728	259		

Table 1: **Selected Tissue Microarrays (TMA) Constructed by OSU**

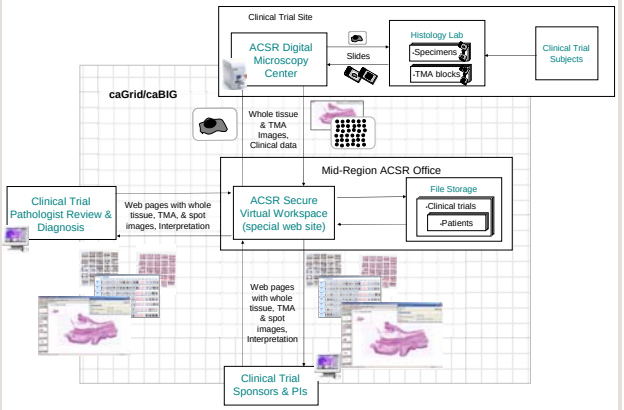

Figure 3: **International/Distant Collaboration based on Tissue Arrays, Digital Images and Internet Communication**

Conclusions

- Digital imaging and *AnalyzeTMA* software facilitated evaluation of stained TMA based tissue sections.
- Samples from resource poor sites were generally troubled by delayed fixation, poor quality formalin fixation, suboptimal tissue processing (particularly temperature control), and poor tissue section H&E stained slide preparation.
- Collected tissues also reflected the difficulty of consistent quality pathology interpretation in resource constrained sites. Lymphomas are particularly troubled because of confusion of lymphoma with other small malignant tumor types and by interpretations attempted from poorly produced H&E stained diagnostic slides.
- Quality performance of globally-gathered FFPET for RNA, DNA, IHC, ISH and FISH excludes some tissues from the research tissue biorepository.

Significance

- QA expedites research, particularly with global collaborators.
- Tissue quality varies as does correctness of pathology designation.
- Support follow up correlative studies by retrospective review of diagnostic biopsy tissue from clinical cancer treatment trials in resource poor settings and cancer epidemiology studies.
- Collaborative digital image review can be a vehicle for quality improvement at resource poor sites.
- Quality performance of globally-gathered FFPET for RNA, DNA, IHC, and ISH and limited DNA integrity should be demonstrated before acceptance into a research tissue biorepository.

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