MULTIMODAL SPECTROSCOPY AS A TRIAGE TEST FOR WOMEN AT RISK FOR CERVICAL NEOPLASIA: HISTOPATHOLOGY REVIEW PROCEDURES AND RESULTS

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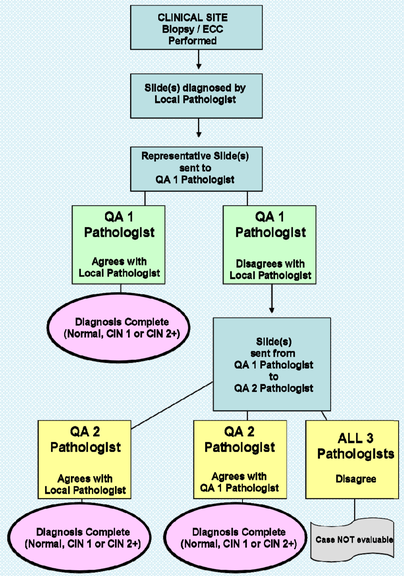
OBJECTIVE: Evaluation of new modalities for the detection of neoplasia requires confidence that the gold standard by which sensitivity and specificity of the new modality are calculated is reliable. For cervical neoplasia, this is especially challenging because the histopathological threshold between cases determined to be positive vs. negative for disease can be subtle, for example the difference between a Cervical Intraepithelial Neoplasia (CIN1) and CIN2 lesion. The objective of the current study was to develop and evaluate a reliable histopathology quality assurance procedure that would aid in the assessment of a new cervical cancer modality, *viz.*, multimodal hyperspectroscopy (MHS).

Figure 1. Quality Assurance Histopathology Flowchart



Pivotal Study Clinical Sites
1-University of Texas Southwest – Claudia Werner, MD / William Griffith, MD
2-Emory University School of Medicine – Lisa C. Flowers, MD / Talaat S. Tadros, MD
3-University of Miami – Leo Twiggs, MD / Nahida Chakhtoura, MD
4-University of Connecticut – Manocher Lashgari, MD
5-University of Arkansas – Alexander Burnett, MD
6-Medical College of Georgia – Daron Ferris, MD
7-Orange County California – Marc Winter, MD / Daniel Sternfeld, MD

METHODS: In this seven-center pivotal study, 1,607 women at risk for cervical neoplasia were tested using MHS (LightTouch, Guided Therapeutics, Inc. Norcross, GA), including 1,456 with abnormal Papanicolaou (Pap) cytology, one with no Pap results and 150 with normal or benign cytology but were at risk for other reasons including positive Human Papillomavirus (HPV) results, previous dysplasia and/or recurrent benign findings (Tables 1 and 2). Histopathologists at each participating center classified tissue samples as belonging to one of three categories: Normal (including metaplasia, inflammation, reparative changes and other benign conditions), CIN1 (including HPV changes and flat condylomas) and CIN2+ (CIN2, CIN3 or carcinoma). Representative slides for all 1,607 subjects were sent to Quality Assurance (QA) Histopathologist 1, Dr. Wilkinson (QA 1). If QA 1 agreed with the diagnosis of the participating site's histopathologist (Site), then for the purposes of the study, the diagnosis was final. If QA 1 disagreed with the diagnosis from the site, the slides were sent to QA Histopathologist 2, Dr. Raab (QA 2). In that case, for each individual tissue sample, agreement by two of the three pathologists was sufficient for final diagnosis. Three way disagreements were considered discordant and if the discordant biopsy had a CIN2+ diagnosis by any pathologist then that subject was considered non evaluable for the assessment of MHS. Each histopathologist was blinded to the diagnosis of the other histopathologists and no review meetings were held to resolve differences in diagnoses. This procedure was initially employed in a previous multicenter evaluation of MHS (DeSantis et al, J. Lower Gen. Tract, Vol 11, no. 1, 2007, 18 - 24). Figure 1 shows the sequence of events leading to the quality assurance histopathology diagnosis.

Table 1. Demographics of study population by study site

Clinical Site	Non Hispanic American Indian Alaska Native	Non Hispanic Asian	Non Hispanic Black or African American	Non Hispanic White	Non Hispanic Native Hawaiian/ Pacific Islander	Hispanic Black or African American	Hispanic White	Non Hispanic TOTAL	Hispanic TOTAL	TOTAL EN-ROLLED
1	0	1	99	27	0	0	107	127	107	234
2	0	2	291	14	1	4	36	308	40	348
3	1	0	89	11	1	4	207	211	102	313
4	0	3	282	22	0	5	82	307	87	394
5	0	1	20	25	0	0	2	46	2	48
6	2	0	84	44	0	0	0	130	0	130
7	0	9	3	107	2	1	18	121	19	140
							TOTAL	1,250	357	1,607

RESULTS: For the 1,607 women in the MHS study, there were 3,302 histopathology samples including 1,885 from biopsies and 1,417 endocervical curettages. For these tissue samples, QA 1's diagnosis agreed with that of the Site 76% of the time (2,524/3,302) and did not require further evaluation by QA 2. Of the 709 samples in which QA 1's diagnosis disagreed with that of the Site, QA 2 agreed with QA 1 51% of the time (365/709) and agreed with the Site 48% of the time (343/709). Three-way disagreements occurred for 2% (70/3,302) of the samples. For determining sensitivity and specificity of MHS, final diagnoses were based on the most severe disease for each of the 1,549 subjects in the study with available histopathology. For these cases, QA 1's diagnosis agreed with the Site 72% of the time (1,110/1,549) and did not require further evaluation by QA 2. Of the 439 cases in which QA 1's diagnosis disagreed with the Site, QA 2 agreed with QA 1 44% of the time (193/439) and agreed with the Site 46% of the time (200/439). Three-way disagreements occurred for 3% (46/1549) of the women (Tables 3 and 4). Of special interest in the evaluation of MHS were biopsies determined to be negative for CIN2+ disease by the Site pathologist and then subsequently found to be positive for CIN2+ disease by both QA histopathologists. For these 38 women QA 1 was required to alert the Site in writing that a possible CIN2+ case had been under diagnosed. (MHS prospectively identified 89% (34/38) of these cases as positive for CIN2+).

CONCLUSIONS: The histopathology QA procedure used in the evaluation of MHS as a new modality in the detection of cervical neoplasia offered several advantages, including blinded diagnoses by three independent histopathologists and a fully prospective method for determining disease outcome for each subject. The trade-off for this was the potential disadvantage that three way disagreements could not be resolved in a histopathology panel setting. However, histopathology agreement rates were high enough (over 70% between the Sites and QA 1 and approximately 50% for the remaining cases) that three-way disagreements leading to non-evaluable cases were kept to a minimum. The procedure also functioned as a method of follow up to determine how MHS performed on cases determined to be negative for CIN2+ by the site but positive for CIN+ upon QA histopathology review.

Table 2. Number and prevalence of final QA histopathology as a function of reason for referral to colposcopy. Excludes subjects with discordant or no histopathology result (n=74).

Reason for Referral	NORMAL	CIN1	CIN2+	TOTAL	% Prevalence CIN1	% Prevalence CIN2+
Negative Pap (Other)*	23	11	2	36	30.5	5.5
ASC/HPV+**	325	272	71	668	40.7	10.6
LSIL	245	330	134	709	46.5	18.9
HSIL	8	26	85	119	21.8	71.4
TOTAL	**601**	**640**	**292**	**1532**	**41.7**	**19.1**

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Table 3. Histopathology agreement by specimens

	Site/QA 1	Site/QA 2	QA 1/QA 2
Total Pathology (per specimen)	3,299	706	706
AGREE (Both Positive)	216	70	79
AGREE (Both Negative)	2,307	272	285
PERCENT AGREEMENT	76%	48%	51%
70 specimens had 3-way disagreement			

Since many subjects had more than one biopsy specimen for diagnosis, each subject's histopathology result was derived from the quality assurance histopathology result for the most severe grade of disease (Table 4).

Table 4. Histopathology agreement by subjects

	Site/QA 1	Site/QA 2	QA 1/QA 2
Total Pathology (per subject)	1,607	383	383
AGREE (Both Positive)	156	57	49
AGREE (Both Negative)	973	150	127
PERCENT AGREEMENT	68%	54%	46%
*53 Subjects had no histopathology / 42 subjects had 3-way disagreement			

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