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Evaluation of ThinPrep™ and SurePath™ ASCUS cases with the Synermed GluCyte™ Thin Layer Methodology

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-ABSTRACT-

Introduction/Purpose: The introduction of liquid based cytology has widely been accepted as an improvement over the conventional Pap Test. Throughout many countries, the high cost of commercially available automated technologies has limited the accessibility of the liquid based technology. New, simpler, and more cost effective manual liquid based thin-layer technologies are being used outside the United States. This study evaluates the ability of one of these technologies, the Synermed GluCyte™ Method, to render and correlate an ASCUS+ diagnosis in previously diagnosed Cytyc ThinPrep™ and Tripath SurePath™ ASCUS cases.

Materials & Methods: The Synermed GluCyte™ Method, using a cell dispersal and adherent solution known as GluCyte™, is a simple and reproducible manual process for thin-layer slide preparation. The reagents are available for non-gynecological use in the USA. For this study, 100 ThinPrep and SurePath residual gynecological specimens diagnosed as ASCUS were obtained. The samples were centrifuged to concentrate the cellular material into a pellet. The supernatant was decanted, the cells were resuspended in GluCyte™ solution, and transferred to conditioned glass slides. After a drying period, staining was performed using a traditional Pap Stain. A circle containing a uniform distribution of cells was then screened by two ASCP certified Cytotechnologists and abnormals were confirmed by a certified Cytopathologist. Bias was prevented and screening proficiency was evaluated by mixing the study samples in with known cases of Negative, LSIL, and HSIL+ prepared using the GluCyte™ Method. Results of the GluCyte™ preparations were compared to the previously diagnosed ThinPrep™ and SurePath™ ASCUS cases. High risk (HR) HPV test results, when available, were used to support a diagnosis in the event a discrepancy existed between the two methods.

Results and Comparisons: The results of the evaluation demonstrated at least equivalency between the two currently widely used methods and the GluCyte™ thin-layer method. Morphologically, the presentations of the various sample components were well represented in the Synermed GluCyte™ Method. ASCUS+ cells presented themselves in similar fashion to both ThinPrep™ and SurePath™ and were readily identifiable by the Cytotechnologists and Cytopathologists. The results of the cytologic comparisons and HPV testing on discrepant cases are represented in tables and some morphologic comparisons can be seen in photomicrographs.

Conclusion: The Synermed GluCyte™ Thin-Layer method offers a more cost effective alternative to currently available liquid based cytology methods. In this study the GluCyte™ method offered at least equivalency to currently available FDA Approved methods in the presentation of diagnostic cells, and allowed for resolution of 82% of ASCUS cases without recourse to HPV testing.

-INTRODUCTION-

Two FDA approved methods for gynecological cytology, Cytyc ThinPrep™ and Tripath SurePath™, have been widely accepted throughout the US as replacements for the conventional Pap smear. Both of these technologies utilize high cost equipment and require expensive disposables. The dependence on expensive components and lack of competition in the US market have led to the higher costs associated with these technologies. Alternatives to these technologies are available for cytology applications outside the US, and for non-gynecological cytology use within the US. One such low cost alternative, known as Synermed GluCyte™ (manufactured in accordance with ISO13485 standards) may have the capability of reducing the cost of gynecologic liquid based cytology without sacrificing the quality of cell presentation that has come to be associated with the automated liquid based cytology methods. Previous studies have evaluated the efficacy of manual liquid based cytology methods and have shown them to be equivalent to the current FDA approved automated methodologies for gynecological cytology[1], and in some cases have allowed for increased disease detection[2]. In this study, we aim to evaluate the cellular appearance of residual liquid based specimens and demonstrate at least diagnostic equivalence of the GluCyte™ method to previously diagnosed ThinPrep™ and SurePath™ cases. In addition, we will evaluate the ability of GluCyte™ prepared slides to resolve previously diagnosed ThinPrep™ and SurePath™ ASCUS cases.

-MATERIALS AND METHODS-

Residual material from four hundred and nineteen (419) previously diagnosed ThinPrep™ and SurePath™ cases were obtained, consisting of Negative, ASCUS, LSIL, and HSIL. Patient identification was removed to blind the study and maintain patient confidentiality. The entire residual sample was transferred to conical centrifuge tubes and centrifuged at 800g for 10 minutes. After centrifugation, the original preservative was decanted, leaving a cell pellet in the centrifuge tubes. Cell pellets were then resuspended using deionized water and vortexed to ensure specimen homogeneity. Next, two drops of the cell suspension were transferred to a round bottom test tube containing 200uL of GluCyte™ Cell Adherent. The GluCyte ™ cell suspension was mixed and 2 drops of this homogeneous cell mixture was transferred to a clean glass slide. The preparation was then allowed to dry into an intact, slide-bound 16-18mm circular transparent membrane, containing diagnostically representative entrapped cells. Slides were then stained using a modified Pap stain. Slides were screened by cytotechnologists and cytopathologists using the diagnostic criteria defined by the current Bethesda system. Bias was prevented by mixing previously prepared and diagnosed GluCyte™ slides with the study samples. Each individual GluCyte™ diagnosis was then compared to the original diagnosis. The ASCUS subset of original diagnoses was then further evaluated by looking at the original high risk HPV results in cases where a 1+ step in diagnostic discrepancies existed. Originally prepared ThinPrep™ and SurePath™ slides were not re-evaluated.

-RESULTS-

Four hundred and nineteen (419) residual ThinPrep™ and SurePath™ samples, including 100 ASCUS cases, were evaluated using preparations made using the Synermed GluCyte™ method. Diagnostic comparisons between the original diagnosis and the GluCyte™ diagnosis can be seen in Table 1. Overall, diagnostic agreement among the Bethesda categories of Negative, ASCUS, LSIL, and HSIL was 87.5%. High risk HPV testing was performed on three LSIL cases that were diagnosed as negative using GluCyte™. Two of the LSIL cases yielded high risk (HR) HPV negative results. In the other case, the HR HPV result was positive and re-evaluation of the GluCyte™ slide did not indicate presence of disease.

Further analysis of the 100 ASCUS cases (58 ThinPrep™, 42 SurePath™) sought to evaluate GluCyte's effectiveness at presenting clinically significant diagnostic cells (see table 1). Of the 100 previously diagnosed ASCUS cases, 26 were classified as Negative, 40 were ASCUS, 31 were LSIL, and 2 were HSIL. High risk HPV results were available on all ASCUS cases and were used to support a specific diagnosis in situations where a diagnostic discrepancy existed between the GluCyte™ and FDA approved automated methodologies (Table 2). Of the 31 cases classified as LSIL using GluCyte™, 27 were HPV HR positive and both of the HSIL cases were also HR HPV positive. In addition, 80% of GluCyte™ negative cases were HR HPV negative. Among ThinPrep™ and TriPath™ cases, 32% and 33% of the preparations were upgraded using the GluCyte™ preparations made from processing residual material.

Table 1

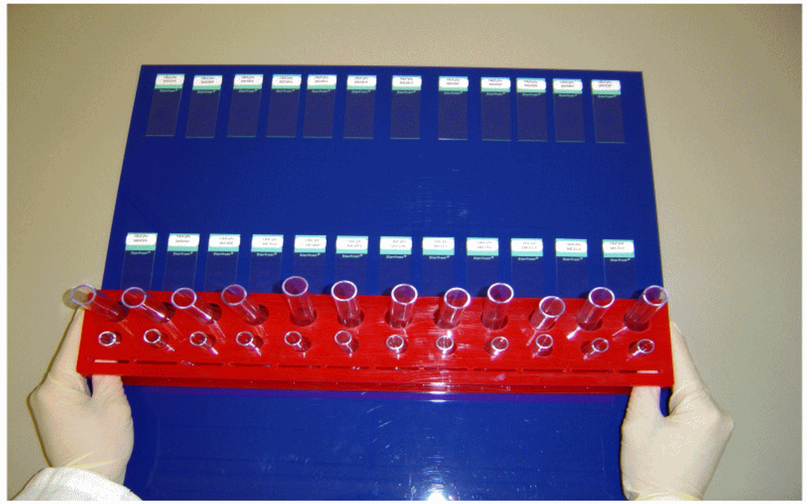
Automated Thin Layer Methodologies

		Negative	*ASCUS*	*LSIL*	*HSIL*	*Totals*
GluCyte™	**Negative**	249	25	3	0	277
	ASCUS	1	42	3	2	48
	LSIL	0	31	43	2	76
	HSIL	0	2	2	14	18
	Totals	250	100	51	18	419

Table 2

Original ASCUS Cases Diagnosed by GluCyte and Paired with HPV HR Result

	Negative, HPV HR-	Negative, HPV HR+	ASCUS, HPV HR-	ASCUS, HPV HR+	LSIL, HPV HR-	LSIL, HPV HR+	HSIL, HPV HR-	HSIL, HPV HR +	TOTAL
ASCUS	20	5	9	33	4	27	0	2	100



-CONCLUSION-

The Synermed GluCyte™ method is an efficient and more cost effective alternative to currently available automated methodologies. The method, although available in the USA for non-gynecologic cytology, has not been approved for use in the USA by FDA as a replacement of the conventional gynecologic Pap smear. The data presented here demonstrate that specimens processed with GluCyte™ will yield diagnoses at least equivalent to currently approved GYN methodologies and more definitive diagnoses in many ASCUS interpretations. The authors believe that the reduction of cell loss and retention of micro-biopsies and diagnostic clusters in the GluCyte™ preparations were key elements in making a more definitive diagnosis in this study. The study demonstrated that preparing a monolayer with GluCyte™ could resolve 82% of ASCUS cases without recourse to HPV testing. In a study by Maksem et al, 90% of 358 ASCUS cases were resolved using manual liquid based cytology[2]. The data of these studies suggest that the Glucyte Method, and other manual methods, may be more effective than current automated liquid based thin-layer procedures at retaining and presenting diagnostic cells and clusters.

-REFERENCES-

1. Dry, J., et al. 2004. Comparison of the New Synermed GluCyte™ Liquid Based Thin-Layer Preparation with both Cytyc ThinPrep™ and TriPath SurePath™ Preparations. ASC Meeting Chicago, Ill.; Poster Presentation 57.

2. Maksem, J., et al. 2005. Resolving ASCUS Without Recourse to HPV Testing: Manual Reprocessing of Residual Automated Liquid-Based Cytology (ALBC) Material Using Manual Liquid-Based Cytology (MLBC). Diagnostic Cytopathology; 33: 434-440.