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Efficacy and Comparison of LG Cocktail and MGG Stain in Air Dried Buccal Smear

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Abstract: ***Leishman Giemsa cocktail is new staining technique, which is used on the air-dried cytology smear. It provide an excellent cytoplasmic and nuclear staining comparable to MGG in both staining quality and diagnostic ability in exfoliative cytology and in the screening program. Cytoplasmic granules were stained better in Leishman Giemsa cocktail and it requires no fixation, procedure will be complete within 10 minute and is less expensive compare to MGG stain. Objectives of the study is to evaluate the quality and staining efficiency of nuclear and Cytoplasmic features by LG stain against MGG.60 slides of buccal smear were collected from 30 students, 30 slides of buccal smear were stained with MGG stain and 30 slides were stained LG cocktail stain. LG cocktail provides better Cytoplasmic transparency and nuclear materials with excellent preservation.***
Keywords*: May-grunwald Giemsa, Leishman-Giemsa cocktail, Buccal smear,Cytology, Romanowsky stain*

I. INTRODUCTION

Staining is a technique that can be used to better visualize cell and cell component under a microscope. By cell components such as a nucleus or a cell wall, or entire cell, most stains can be used on fixed or non-living cells while only some can be used on either living or non-living cells.[1]Routinely various stains like H&E, Romanowsky and PAP had been used in Cytology[2]. PAP stain is the universal stain used for staining PAP test slide. The papanicolaou method is a polychrome counter-stain method based on dye competition in the Cytoplasm combined with a nuclear haematoxylin staining. Blue colour of the nucleus is enhanced by alum. Orange-G eosin alcohol, light green, and Bismarck-brown are the Cytoplasmic stain.[3,4].Leishman stain is used for staining the blood Film, these stain allow better estimation of cell size, nuclear size, cell Cytoplasmic features but not nuclear chromatin. It's a good nuclear stain which is widely used in haematology lab[5]. MGG is a modification of Geimsa stain. This is routinely used in cytology for air-dried smear in many laboratories. This stain demonstrates the Cytoplasmic features but not the nuclear chromatin well and the procedure is time consuming and stain has tendency to precipitateand is stain nucleus as blue/ violet colour cytoplasm as pink and bacteria as blue[6] . Leishman Giemsa cocktail is new staining technique, which is used on the air-dried cytology smear. It provide an excellent cytoplasmic and nuclear staining comparable to MGG in both staining quality and diagnostic ability in exfoliative cytology and in the screening program.[7]Cytoplasmic granules were stained better in Leishman Giemsa cocktail and it requires no fixation, procedure will be complete within 10 minute and is less expensive compare to MGG stain. This cocktail can be used for staining routinely of air-dried smears to provide good staining quality, which adds overall efficacy to the result.

II. AIM AND OBJECTIVES

A. Aim

To study and evaluate the diagnostic efficiency and reliability of Leishman Giemsa (LG) cocktail in comparison with May-Grunwald (MGG) stains in air-dried buccal smears.

B. Objectives

1) To evaluate the quality and staining efficiency of nuclear and Cytoplasmic features by LG stain against MGG.
2) To check the artifacts formation in LG and MGG stain.
3) To evaluate the amount of cellularity after staining with LG and MGG stain.

III.MATERIALS AND METHODS

A total of 30 students in the department of MLT including in this study 60 buccal smear were collected from 30 students. One of the smear were stained with MGG and other with LG cocktail stain.

A. Inclusion Criteria

Buccal smear of healthy individual were selected between 18 to 30 years with no history of habits tobacoo smoking, betel chewing and alcohol consumption.

B. Exclusion Criteria

1) Cases where the biopsy procedure was carried on oral cavity.
2) Patients with systemic disorders and uncooperative patients.
3) Oral ulceration
4) Oral carcinoma

C. Methods of Collection of Sample

Written consent was obtained from all the students participated in this study. Explain the simplified procedure of the buccal smear collection. Before samples were taken, rinse their mouth with several changes of water to eliminate debris and excess saliva from the oral mucosa., with the help of a wooden stick, Sample were spread on a clean, grease free, per-numbered glass slide and allow to air-dry the smear mucosa exfoliative epithelial cells were obtained from right and left buccal .

D. Method for Processing of Samples

For this study to be conducted ,60 samples were taken in total, with 30 smears for MGG stain and 30 smears for L-G stain obtained from students. For MGG staining an air dried smear is prepared. Place the air dried smear in May-Graunwald stain for 5 min. Wash the slide in running tap water about 5to10 dips. Then place the slide in Giemsa stain for 5 min. Air dried the smear. For LG cocktail staining, first LG cocktail is prepared by mixing Leishman and Giemsa equal amount.Air dry the smear Add the L&G cocktail to cover the smear for 3 min. Then add the phosphate buffer to speared the whole area of slide for 5 min. Wash with water. Dry the smear. The stained Cytosmear were viewed under the compound light microscope and Cytopathologically graded based on the criteria given by Von Hamm [8]. The slides were analysed for four parameters.

TABLE I

PARAMETER	QUANTITATIVE DESCRIPTION	POINT SCORE
Background	1.Intensely stained obscuring cellular details	Satisfactory
	2.Moderately stained with better celluar details	Good
	3.Less intense staining with crisp cellular details	Excellent
Amount of cellular material	1.Minimal to absent:Diagnosis not possible	Satisfactory
	2.Sufficient for cytodiagnosis	Good
	3.Abundant:Diagnosis simple	Excellent
Nuclear details	1.sumudgy	Satisfactory
	2.Fair preservation but chromatin granularity not appreciable	Good
	3.Excellent preservation with crisp chromation	Excellent

Cytoplasmic details	1.Non-transparent masking of nuclear details	Satisfactory
	2.Non-transparaent with intact cell membrane	Good
	3.Transparent intact cell membrane without masking of nuclear details	Excellent

IV. RESULT

The present study was undertaken to compare and evaluate the efficacy of LG stain, MGG stain in cytological diagnosis. For normal group, we observe background, amount of cellular materials, nuclear materials, cell cytoplasm.In background we observe 14(46%) LG cocktail slides and 12(40%) MGG slides with intensely stained obscuring cellular details. And we observe 9 (30%) LG cocktail Slides and 14(46.6%) MGG slides with moderately stained with better cellular details. And we observe 7 (23%)LG cocktail slides and 4 (13.3%)MGG Slides with less intense staining with crisp cellular details. For the amount of cellular material we observed 4(13%) LG cocktail slides and 7 (23%) MGG slides with minimal to absent diagnosis not possible And we observe 10(35%) LG cocktail slides and 9 (30%) MGG slides with sufficient for cytodignosis. And we observe 16(53.3%) LG cocktail slides and 14 (46.6%) MGG slides with abundant diagnos is simple. In nuclear details we observe 3(10%) LG cocktail slides and 3 (10%) MGG Slides with smudgy .And we observe12(40%) LG cocktail slides and 19(63.3%) MGG slides with fair preservation but chromatin granularity not appreciable. And we observe 15 (50%) LG cocktail slides and 8(26.6%) MGG Slides with excellent preservation with crisp chromation . Qualitative analyses of the cytosmear obtained in study cases showed nuclear details were better in LG cocktail than MGG staining. In cytoplasm we observe 4 (13%) LG cocktail slides and 10 (35%) MGG slides with non –transparent masking of nuclear details. And we observed 8 (26%) LG cocktail slides and 17 (56%) MGG slides with non-transparent with intact cell membrane. And we observe 18(60%) LG cocktail slides and 3(10%) MGG slides with transparent, intact cell membrane without masking of nuclear details. cytoplasmic staining is better in LG cocktail than MGG staining.

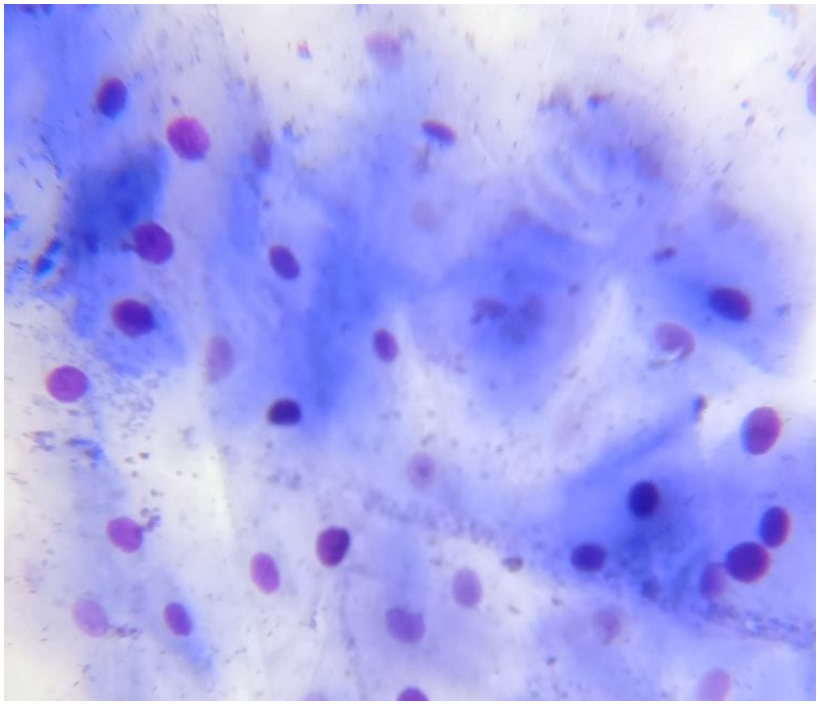

Fig 1 MGG staining

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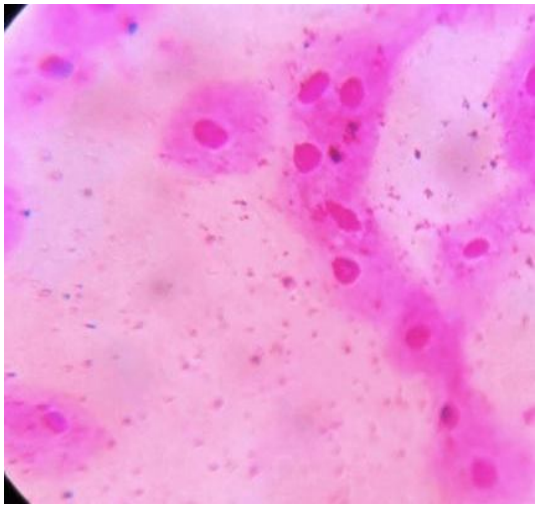

Fig 2 LG cocktail staining

Table 2: Comparison of background staining with LG cocktail stain and MGG.

Staining	Satisfactory	Good	Excellent
LG	14 (46%)	9 (30%)	7 (23%)
MGG	12 (40%)	14(46.6%)	4 (13.3%)

Table 3 :Comparison of Amount of cellular materials staining with LG cocktail stain and MGG.

Staining	Satisfactory	Good	Excellent
LG	4 (13%)	10 (35%)	16 (53%)
MGG	7(23%)	9 (30%)	14 (46%)

Table 4 : Comparison of nuclear materials staining with LG cocktail stain and MGG stain .

Staining	Satisfactory	Good	Excellent
LG	3 (10%)	12 (40%)	15 (50%)
MGG	3 (10%)	19 (63.3%)	8 (26.6%)

Table 5 : Comparison of cell cytoplasm staining with LG cocktail stain and MGG.

Staining	Satisfactory	Good	Excellent
LG	4 (13%)	8 (26%)	18 (60%)
MGG	10 (35%)	17 (56%)	3 (10%)

V. DISCUSSION

In our study 60 buccal smears were collected from healthy students and 30 smears where stained by LG cocktail stain and MGG stain. The qualitative assessment of 2 stains were done by scoring under four different parameters, they are background, amount of cellular materials, nuclear materials and Cytoplasm details.A study by Maumita Bhattacharaya etal is “comparison of efficacy and reliability of different histochemical stain in oral exfoliative Cyt. A qualitative analysis”. In this study they use 4 stains such as PAP, H&E, MGG, LG cocktail stain. For vivid meta chromatic staining of certain Cytoplasmic products, stromal, and background elements, many Cytologist prefer Romanowsky stains over PAP, H&E for FNAC specimens and also the air dried smear.MGG stain with high background staining obscure background material and also the cellular details. Therefor MGG needs preparation of fresh solution everyday[6]. The qualitative assessment of stain was done by scoring four different parameters for each stain. kappa statistics were applied to measure the agreement between three observed over qualitative parameters.

On correlating the Cytological diagnosis , histopathology report, MGG show statistical significant score (69.7%) depicting both are equally good with equal score, and they have ability to tell correctly and probability that person with negative test result do not have the disease. A study by Supreet k. Sidhu etal is "comparing the Efficacy of Leishman-Giemsa cocktail stain Giemsa stain and Papanicolaou stain in potentially malignant oral lesion :A study on 540 Cytological sample" .In this study they use four stain Rapid Pap, Giemsa stain, LG cocktail .The study participants comprised 540 smear taken from 180 participants, including 60 normal patients as control ,60 potentially malignant disorder in oral region, and evaluated the efficacy of the three stain. Belgaumi and Shetty performed a study on 100 healthy control and 100 patient diagnosed with squamous cell carcinoma. Garbyal etal analysed 720 cases in their study[2].A study by Sunethri Padma etal,is "A comparative study of staining characteristics of Leishman-Geimsa cocktail and papanicoaou stain in cervical Cyt".In this study they use two stains, they are PAP and LG cocktail. In this study LG cocktail stain has given good staining to the nucleus in 60% of the smears. The findings were similar to that of supreet K sidhu etal[10]. LG cocktail is staining cervical smears and is proved to be better than the routine stains .There result accordance to study by Belgaumi and Shetty who also observed statistically significant differences when the Cytoplasmic staining was compared for pap and Giemsa Vs. LG . Hence, from the above data ,we found that LG stain was more efficient in Cytoplasmic and nuclear staining in comparison with other stain. Which is in accordance with the study of Garbyal etal. and Mitra etal. LG is also superior to Giemsa stain both in staining characteristics.[2]

VI. CONCLUSION

In my study, Efficacy and comparison of LG cocktail and MGG stain in air dried buccal smear. I Concluded that LG cocktail come out better than MGG stain

A. LG cocktail provide rapid diagnosis and minimum staining time.
B. LG cocktail provide better background details with minimum artefact.
C. Both MGG and LG cocktail shows better amount of cellular materials.
D. LG cocktail provide better nuclear materials with excellent preservation with crisp chromatin than MGG stain.
E. LG provide better Cytoplasmic transparency without masking of nuclear details than MGG stain.

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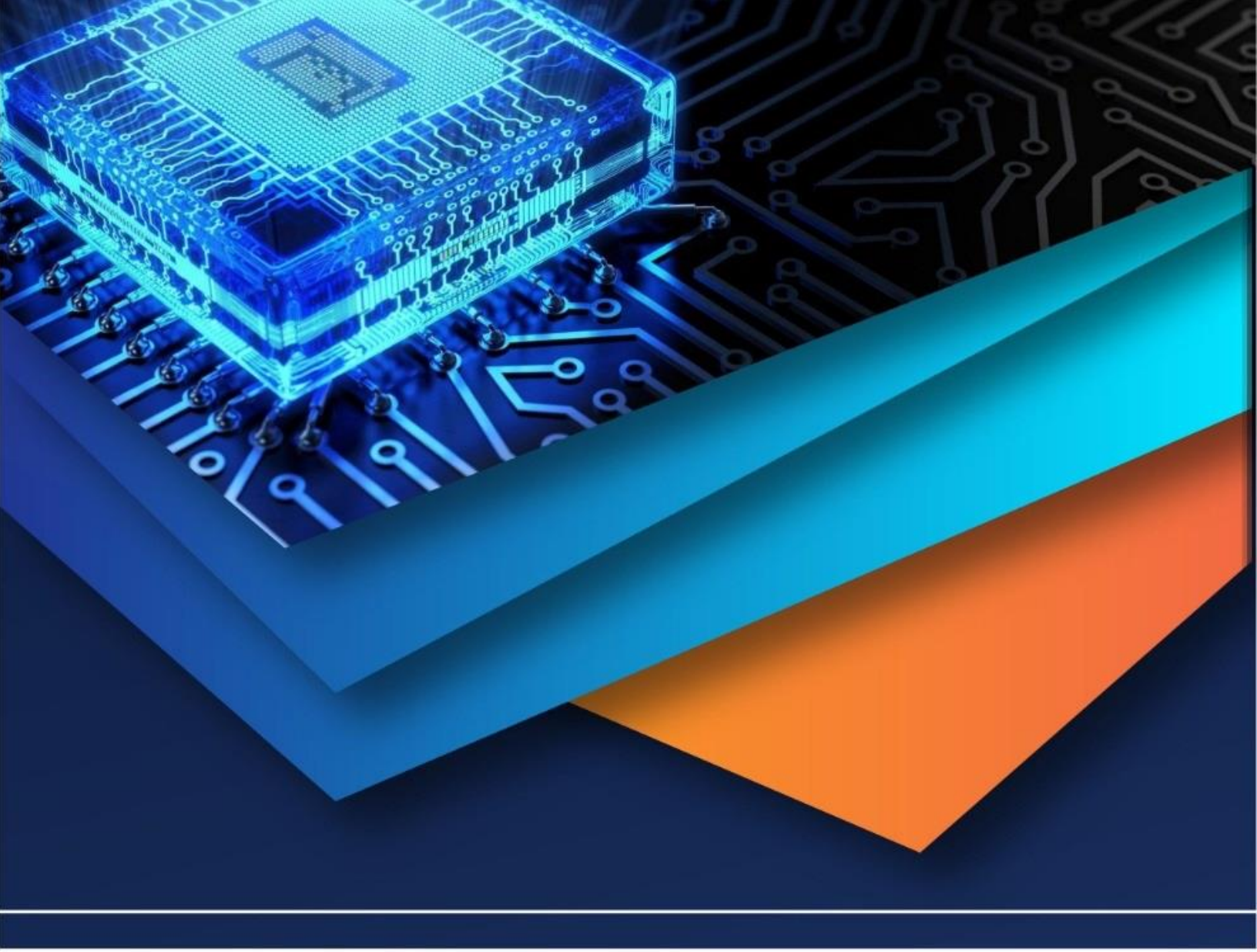
REFERENCES

[1] Monica Z. Bruckner, Montana ;microbial life educational resources.
[??] Garbyal RS, Agarwal N, Kumar P. Leishman-Giemsa cocktail: An effective Romanowsky stain for air dried Cytologic smears. Acta Cytol. 2006;50:403–06
[??] Marluce Bibbo;David C Wilbor4:Mathilde E. Boon and Albert J.H. suurmeijer; The PAP smear; third Edition; harwood academic publishers ;page number 257
[??] Rana S. Hoda, MD, FIAC, SA Hoda; Fundamentals of pap Test Cyt; Springer Sciences and Business Media, 2009.
[??] Suryalakshmi S, Thangamani P, Ravi S, Niveditha T, Kumar IVS, Premalatha, et al. Comparision of leishman staining with H&E staining technique in the study of FNAC smears. International Organization of Scientific Research. 2016;15:34–395.
[??] Belgaumi UI, Shetty P. Leishman Giemsa cocktail as a new, potentially useful Cytological technique comparable to papanicolaou staining for oral cancer diagnosis. Journal of Cyt. 2013;30(1):18–22
[??] Geisinger KR, Stanley MW, Raab SS, Silverman JF, Abati A. Modern Cytopathology. 1st ed.
[??] Rajendran R, Shivapathasundaram B. Shafer's Textbook oral pathology.6th Ed.philadelphia:Elesvier.2010.p.112.
[??] Koss LG. Cytologic techniques, Diagnostic Cyt and its histopathologic basis, Carol E Bales. (5th edition) 2006 ;Vol.2:1592–1601
[??] Supreeet K Sidhu etal jouranal of Cyt:2018;35(2)105-109
[11] M. Metev and V. P. Veiko, Laser Assisted Microtechnology, 2nd ed., R. M. Osgood, Jr., Ed. Berlin, Germany: Springer-Verlag, 1998.
[12] SJ. Breckling, Ed., The Analysis of Directional Time Series: Applications to Wind Speed and Direction, ser. Lecture Notes in Statistics. Berlin, Germany: Springer, 1989, vol. 61.
[13] S. Zhang, C. Zhu, J. K. O. Sin, and P. K. T. Mok, "A novel ultrathin elevated channel low-temperature poly-Si TFT," IEEE Electron Device Lett., vol. 20, pp. 569–571, Nov. 1999.

[14] M. Wegmuller, J. P. von der Weid, P. Oberson, and N. Gisin, "High resolution fiber distributed measurements with coherent OFDR," in Proc. ECOC'00, 2000, paper 11.3.4, p. 109.
[15] R. E. Sorace, V. S. Reinhardt, and S. A. Vaughn, "High-speed digital-to-RF converter," U.S. Patent 5 668 842, Sept. 16, 1997.
[16] (2002) The IEEE website. [Online]. Available: http://www.ieee.org/
[17] M. Shell. (2002) IEEEtran homepage on CTAN. [Online]. Available: http://www.ctan.org/tex-archive/macros/latex/contrib/supported/IEEEtran/
[18] FLEXChip Signal Processor (MC68175/D), Motorola, 1996.
[19] "PDCA12-70 data sheet," Opto Speed SA, Mezzovico, Switzerland.
[20] Karnik, "Performance of TCP congestion control with rate feedback: TCP/ABR and rate adaptive TCP/IP," M. Eng. thesis, Indian Institute of Science, Bangalore, India, Jan. 1999.
[21] J. Padhye, V. Firoiu, and D. Towsley, "A stochastic model of TCP Reno congestion avoidance and control," Univ. of Massachusetts, Amherst, MA, CMPSCI Tech. Rep. 99-02, 1999.
[22] Wireless LAN Medium Access Control (MAC) and Physical Layer (PHY) Specification, IEEE Std. 802.11, 1997.



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