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New Decontamination and Concentration Technique Decomics® and LED Microscopy: Excellent and Rapid Mycobacterial Detection Tools for Smear Microscopy

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A B S T R A C T

Smear microscopy still remains the bedrock of tuberculosis (TB) diagnosis, in spite of innumerable advances, particularly in developing countries. So far, ZN microscopy is the most expended amongst all methods currently employed in laboratories for diagnosis on account of its simplicity, speed, low cost, and minimal requirement of equipment[1] and technical skills despite the fact that LED microscopy is a better diagnostic tool. WHO, also recommends that that LED microscopy be phased in as an alternative for conventional ZN light microscopy.[2] So the present study was planned with the aim of evaluating the efficacy of LED Microscopy and a new inspiring technique "Decomics®" kit, developed and tested by Salubris Inc USA, based on absorbent beads, which does not require centrifugation and is easy to use and swift. This is the first study in India utilizing Decomics® method. A cross sectional study was conducted on 500 sputum samples from clinically suspected cases of pulmonary tuberculosis. Among the 500 clinically suspected cases of pulmonary tuberculosis, *Mycobacterium tuberculosis* was detected in 100 cases (20%) by both direct microscopes. Where LED microscopy gave 100% result and direct ZN light microscopy obtained 90% positivity. Efficiency of "Decomics®" as decontamination in detection of mycobacteria was better than classical and popular NaOH-NALC. Elimination of need for centrifugation decreased processing time by half and higher detection rate of mycobacteria were important advantages of Decomics®.

Introduction

Smear microscopy still remains the bedrock of tuberculosis (TB) diagnosis, in spite of innumerable advances, particularly in developing countries. So far, ZN microscopy is the most expended amongst all methods currently employed in laboratories for diagnosis on account of its simplicity, speed, low cost, and minimal requirement of equipment[1] and technical skills despite the fact that LED microscopy

is a better diagnostic tool. WHO, also recommends that that LED microscopy be phased in as an alternative for conventional ZN light microscopy.[2]

In India, till date RNTCP relies on direct sputum smear microscopy for diagnosis, concentration of the sample will act as abene factor. As Concentration not only aids in the detection but also increases the positivity rate when compared to direct microscopy of any sample even amongst paucibacillary. However, routinely used concentration methods can increase the risk of specimen cross-contamination and occupational transmission to laboratory workers.[3] Thus decontamination and concentration are used collectively.

Grading of smear yields prognostic as well as diagnostic value, for early diagnosis and judging prognosis. A very few studies have undertaken the concept of evaluating the grading of smears after decontamination and concentration procedures.

So the present study was planned with the aim of evaluating the efficacy of LED Microscopy and a new inspiring technique "Decomics®" kit, developed and tested by Salubris Inc USA, based on absorbent beads, which does not require centrifugation and is easy to use and swift. This is the first study in India utilizing Decomics® method.

Materials and Methods

A cross sectional study was conducted on 500 sputum samples from clinically suspected cases of pulmonary tuberculosis between September 2012-September 2014 Total of 2 yrs duration in Department of Microbiology, MMIMSR Mullana Ambala after Ethical clearance was taken from ethical committee. The specimen was split approximately into two equal parts and was processed by digestion, decontamination and concentration by NaOH-NALC procedure as per standard protocol (ref) and by Decomics kit® [manufactured & developed by Salubris Inc. Decomics consists of three components: 1.Sample cup containing decontamination solution with pH indicator 2. Absorbent beads; 3. Neutralization solution. 2.0ml sputum was dispensed into the sample cup containing decontamination solution and left for 10 minutes, absorbent beads were then added and the cup was vortexed for 30 seconds and kept till the time the crackling of beads stops, (3-5 mins), making total decontamination duration 15 minutes. Neutralization solution was subsequently poured in the cup and held for 5 minutes. Smears were prepared from processed samples after both NaOH-NALC and Decomics and subjected to ZN light microscopy and LED fluorescent microscopy.

Statistical analysis of the study was done using fisher's test.

Results and Discussion

Among the 500 clinically suspected cases of pulmonary tuberculosis, *Mycobacterium tuberculosis* was detected in 100 cases (20%) by both direct microscopes. Where LED microscopy gave 100% result and direct ZN light microscopy obtained 90%positivity (Table.1). LED Microscopy proved to be better in all aspects as compared to ZN Light microscopy as on examination of direct smear as compared to ZN light microscopy it showed 2% more positivity. Concentration and decontamination techniques NaLC-NaOH and Decomics increased the smear positivity from 18% (direct microscopy) to 20% and 21.2% with NaLC-NaOH and Decomics respectively by ZN microscopy. LED smears positivity also increased after

concentration and decontamination from 20% to 22% and 23.2% with NaLC-NaOH and Decomics® respectively. Decomics® indicated 1.2% more positivity when compared to NALC-NaOH by both Zn as well as LED microscopy as depicted in table no.2.

Concentration and decontamination methods also have the potential of extracting positivity even in the smear negative cases. 2.4% and 3.9% positivity was seen amongst smear negative samples after decontamination with NaLC-NaOH and Decomics® respectively by ZN Light Microscopy (Table.3). And2.5% and 5% positivity was seen in smear negative samples after decontamination with NaLC-NaOH and Decomics® respectively with LED microscopy (Table.4). Indisputably Decomics came out to be a better technique in tuberculosis detection as compared to NALC-NaOH when evaluated by both ZN and LED microscopy.

Grading of smears acts as both diagnostic as well as prognostic gauge for tuberculosis patient. Decontamination and concentration procedures elucidate their effect on the grading of smears. There was weighty increase in positivity after decontamination. In ZN Microscopy, a decrease in the number of scanty samples from 10% on direct microscopy to 5% after NaLC-NaOH and 3% after Decomics® while in LED microscopy number of scanty samples decreased from 7% on direct microscopy to 3.6% and 2.5% after NaLC-NaOH and Decomics® respectively. In contrast Number of grade 3+ smears increased from 37 in direct microscopy to 43 after NALC and 43 after Decomics with Zn microscopy while on LED microscopy from 42 cases on direct microscopy to 49 after NALC and 52 after Decomics®.

Case detection is an important module of the DOTS strategy as encouraged by the WHO. The backbone of TB diagnosis worldwide continues to be smear microscopy especially in resource-poor settings.[4] Where the TB burden is greatest &the value of any diagnostic procedure depends on its costs and efficiency.

Excellent sensitivity and specificity, the short evaluation time which can be ascribed to quicker scanning of each field because of increased visibility of the Mycobacteria; the decreased magnification used during fluorescence microscopy, compared with light microscopy also contributes, and also simplicity of the fluorochrome staining method, compared with ZN method.[5] WHO stated Qualitative assessments of LED microscopy confirmed many anticipated advantages, including use of the devices without a dark room, durability and portability (in the case of attachment devices); user acceptability in all field studies was reported as excellent.[2]

A difference of 10% sensitivity was seen in the present study when both the microscopes were compared (Table I) (p value= 0.6138 which was not statistically significant) (Sensitivity 89.8%, Specificity 99.5%,Positive PPV 97.8%,NPV 97.55) which is in accordance with Cuevas *et al.,* [6] and Albert *et al.,* (2013) [7]. Whitelaw A *et al.,* in their study depicted that Fluorescent stains increase sensitivity by >10% over Carbol fuchsin-based stains and reduce the time required to read smears.[8]

Table.1 Rate of positivity by DIRECT microscopy

Total cases Studied	Total positivity by microscopy	ZN microscopy	LED Microscopy
500	100(20%)	90(90%)	100(100%)

Table.2 Rate of smear positivity with both ZN and LED Microscopy before and after decontamination.

Total No. of samples studied	ZN			LED		
	Before Contami -nation	After Contamination		Before Contami -nation	After Contamination	
	Direct	**NaLC-NaOH**	**Decomics®**	**Direct**	**NaLC-NaOH**	**Decomics ®**
500	**90 (18%)**	**100 (20%)**	**106 (21.2%)**	**100 (20%)**	**110 (22%)**	**120 (23.2%)**

Table.3 Rate of detection of *Mycobacterium tuberculosis* by **ZN Staining** Before and After Decontamination

DIRECT Smear status	Before Decontamination	After decontamination	
		NaLC -NaOH	**Decomics®**
SMEAR +VE SAMPLE	90	90(100%)	90(100%)
SMEAR – VE SAMPLE	410	10(2.4%)	16(3.9%)
TOTAL	**500**	**100(20%)**	**106(21.2%)**

Table.4 Rate of detection of *Mycobacterium tuberculosis* by LED Microscopy Before and After Decontamination

DIRECT Smear Status	Before Decontamination	After decontamination	
		NaLC-NaOH	**Decomics®**
SMEAR +VE SAMPLE	100	100(100%)	100(100%)
SMEAR –VE SAMPLE	400	10(2.5%)	20(5%)
TOTAL	**500**	**110(22%)**	**120(23.2%)**

Table.5 Overall positivity of *Mycobacterium tuberculosis* by microscopy, Decontamination techniques & smear grades.

Grades	ZN Microscopy			LED		
	Direct	**NaLC-NaOH**	**Decomics®**	**Direct**	**NaLC-NaOH**	**Decomics ®**
3+	37(41.1 %)	43(43%)	43(40.5%)	42(42%)	49(44.5 %)	52(43.33 %)
2+	25(27.8 %)	32(32%)	34(32.07%)	30(30%)	32(29.0 9%)	36(30%)
1+	19(21.1 %)	20(20%)	25(23%)	21(21%)	25(22.7) %	29(24.16 %)
Scanty	9(10%)	5(5%)	4(3%)	7(7%)	4(3.6)%	3(2.5%)
Total	90	100	106	100	110	120

As a simple and rapid test, sputum smears are frequently adopted to evaluate infectivity and treatment effectiveness. Decontamination and concentration of sample still now is majorly used in isolation of TB bacilli, but it also aids in increasing positivity of smears thus a combination of these two, used in present study would help to achieve amplified, precise and brisk TB diagnosis by smear microscopy. To help microscopy achieve the status of a crucial index present study used a newer method called the "Decomics®" developed and tested by Salubris Inc USA, based on absorbent beads, which have pores smaller than bacteria, absorb most of the solutions and leave 2-3ml concentrated sample behind.[9]

Decomics® excluded the tedious centrifugation process. All current decontamination and concentration methods require centrifugation, which not only is expensive for resource-limited settings but also poses risks to the laboratory workers during processing because of airborne droplets released. Decomics was found to be very efficient and less time consuming than the most popularly implied method NaLC-NaOH, it consumed around15-25 minutes. Sample processing was safer and easier with it since the whole procedure was completed in the same cup.

Present study revealed a considerable difference in microscopic examination by both ZN and Led microscopes after concentration and decontamination procedures (Table.2) which is in accordance to Hooja, *et al.*,[10] in their study that the sensitivity increased by 6.67% for ZN Microscopy after concentration and decontamination. And also by Morcillo, N. *et al.*, [1] who showed an increase of around 2.2% after concentration in comparison to that of specimens without concentration. Also present study revealed better performance of LED microscopy in detection as compared to ZN both before and after decontamination and concentration. The efficiency of Decomics in extraction of mycobacteria from clinical samples was undeniably better than classical NaOH-NALC.

Paucibacillary samples have always been a woe for microscopy as they usually present negative on smear. Delays in diagnosis have a significant impact on the patient by increasing the risk of severe disease and mortality.[11] Decontamination and concentration procedures break down the

cell releasing the intracellular bacilli outside thereby increasing the positivity rate on microscopy. When evaluated the direct smear negative samples after decontamination and concentration procedures in the present study there was an increase in positivity of smears. Decomics® and LED microscopy combination again proved to better in detecting mycobacteria in smear negative samples (Table.3, 4).

Grading of the smears gives an idea regarding the bacterial load. It depends upon various factors such as time of collection, number of samples taken, nature of sample, antitubercular treatment, observer's competency etc. Infectiousness of tuberculosis correlates with the number of bacilli in the sputum (reflected by the sputum grading) and the length of infectiousness and very likely increasing the probability of transmission of infection in the community. Present study elucidated a change in the pattern of grading of the positive smears after decontamination and concentration where Decomics again increased the grading of a substantial number of scanty samples and also an increase was seen in 3 + samples.

The Present study, LED Microscopy after fluorescent staining yielded better results than the conventional ZN staining and is recommended by WHO too, but it comes with a drawback of being evaluated by LED Microscope which is expensive and requires an expert eye. Efficiency of "Decomics®" as decontamination in detection of mycobacteria was better than classical and popular NaOH-NALC. Elimination of need for centrifugation decreased processing time by half and higher detection rate of mycobacteria were important advantages of Decomics®. Concentration procedures also proved to be an efficient tool in extracting positivity in smear negative cases thus could help clinicians to provide prompt treatment. Use of a combination of Decomics® and LED Microscope can be a boon in rapid diagnosis of pulmonary tuberculosis.

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