

Role of Agnor Staining in Diagnosis of Malignant Serous Effusions – A Cross-Sectional Study

Akhila R¹, Vinayraju D², Gururaju D³, Ramu R⁴

¹Senior Resident, ⁴Professor, Department of Pathology, Basaveshwara Medical College, and hospital, Chitradurga, Karnataka, India. ²Gynecologist, CHC Bharamsagara, Chitradurga Taluq & District, Karnataka, India. ³Assistant Professor, Department of Pediatrics, CMCRI Chitradurga, Karnataka, India

Abstract

Background: Serous effusions are the fluids accumulated in body cavities like the pleural, pericardial, and peritoneal cavities. Detection of malignant cells in these effusions helps in staging and planning the course of management. Accurate identification of the malignant cells using conventional cytological examination is a diagnostic challenge. Argyrophilic nucleolar organiser regions (AgNORs) are a simple cytological technique that is useful for detecting these malignant cells in effusions. Hence, in the present study, the silver staining method is applied to differentiate malignant cells from reactive mesothelial cells in serous effusions. The objective is to determine the significance of AgNOR staining in differentiating benign and malignant serous effusions and to determine the prevalence of malignant changes in serous effusions. **Material and Methods:** This is a sectional study conducted from August 2022 to December 2023 in the Department of Pathology at Basaveshwara Medical College and Hospital. This study includes serous effusions sent to the central lab for cytological examination. All samples were centrifuged, and smears were prepared and stained with H&E and AgNOR. AgNORs are counted as black dots in the nuclei of 100 cells using 100x oil immersion. The pattern of AgNOR dispersion and its shape will be compared in reactive and malignant cases. **Results:** Out of 310 samples, 176 were ascitic, and 134 were pleural fluids. Prevalence of malignancy in these samples was 11.95%. Mean agnor count in NFM was 2.33 +/- 0.67, AUS was 5.68 +/- 1.99, SFM was 7.87 +/- 2.25, and in malignancy was 11.04 +/- 0.9, indicating that the mean count is higher in malignant cells than in benign cells. Benign cells had Agnor dispersion of 0-1+, and malignant cells had dispersion of 2+- 3+. Similarly, benign cells had an average size of 0-1+ and malignant cells had an average size of 2+-3+. Agnor size had a sensitivity of 91.89% and a specificity of 97.76% in detecting malignant cells. Agnor dispersion had a sensitivity of 94.59% and a specificity of 99.1% in detecting malignant cells. **Conclusion:** Mean Agnor count is significantly elevated in malignant cells when compared to benign cells. Along with increased size and dispersion of Agnor, a count is seen in malignant cells of serous effusion. Agnor stain is a particularly useful and rapid diagnostic test for differentiating benign from malignant cells when routine cytology fails.

Keywords: AgNor staining, serous effusions: Agnor size, Agnor dispersion.

Received: 18 November 2025

Revised: 08 December 2025

Accepted: 30 December 2025

Published: 23 January 2026

INTRODUCTION

Serous effusions are the fluids accumulated in body cavities like the pleural, pericardial, and peritoneal cavities. These serous effusions can be transudate or exudate. Varieties of conditions, ranging from inflammatory to neoplastic, are responsible for these effusions. Malignancies can cause effusions by hematogenous spread or direct invasion.^[1] Detection of malignant cells in these effusions helps in staging and planning the course of management. Accurate identification of the malignant cells using conventional cytological examination is a diagnostic challenge.^[2] The sensitivity of traditional cytology for detecting malignant cells ranges from 50% to 78%.^[3] Other methods, such as immunohistochemical stains, electron microscopy, and DNA flow cytometry, yield better results but are expensive and time-consuming; moreover, some antigens used in these methods can be expressed by both normal and reactive proliferative mesothelial cells.^[4]

Argyrophilic nucleolar organiser regions (AgNORs) are a simple cytological technique that is useful for detecting these malignant cells in effusions. Nucleolar organiser regions (NORs) are loops of DNA located on acrocentric

chromosomes and can be detected by silver staining.^[5] These NORs are associated with argyrophilic proteins, which are increased in malignant cells. The argyrophilic associated proteins are nucleolin and nucleophosmin. On silver staining, these argyrophilic proteins appear as black dots. The number and size of these NORs help to distinguish between benign and malignant cells.^[6] Hence, in the present study, the silver staining method is applied to differentiate malignant cells from reactive mesothelial cells in serous effusions.

Objectives of the Study

1. To determine the significance of AgNOR staining in differentiating benign and malignant serous effusions.

Address for correspondence: Dr. Vinayraju D,
Gynecologist, CHC Bharamsagara, Chitradurga Taluq & District, Karnataka, India.
E-mail: vinayraju319@gmail.com

DOI:
10.21276/amt.2026.v13.i1.306

How to cite this article: Akhila R, Vinayraju D, Gururaju D. Role of Agnor Staining in Diagnosis of Malignant Serous Effusions – A Cross-Sectional Study. Acta Med Int. 2026;13(1):113-118.

2. To determine the prevalence of malignant changes in serous effusions.

MATERIALS AND METHODS

Source of data: This hospital-based cross-sectional study was conducted between Aug 2022 and December 2023 in the department of pathology in a tertiary care hospital in Chitradurga.

Inclusion criteria: All patients presenting with pleural, peritoneal, and pericardial effusions and aspirated samples sent to the central laboratory, department of pathology for cytological examination.

Exclusion criteria: Serous effusions in patients with known malignancy who are on treatment.

Sample size was calculated using the formula $4pq/d^2$, with a prevalence of 11% and an absolute precision of 5%. The sample size is 157

Methodology: All samples received were centrifuged within 2 hours of aspiration. The centrifugation was performed at 2000 rpm for 5 minutes. Four smears were prepared from sediment, immediately fixed in 95% ethanol, and two were stained with H and E, and the other two with AgNOR stain.

H and E STAIN: After staining with H and E, slides were visualized under a light microscope (LABOMED Lx500). They were classified based on the International System for Reporting Serous Fluid Cytopathology (TIS) into five categories, namely: Non-Diagnostic (ND), Negative for Malignancy (NFM), Atypia of Undetermined Significance (AUS), Suspicious for Malignancy (SFM), Malignant (MAL).^[7]

AGNOR STAIN: AgNOR staining solution is prepared by dissolving 2 g of gelatin in 1% aqueous formic acid to make a 100 ml solution at a concentration of 2 % (solution A). A fifty percent aqueous silver nitrate solution was prepared by dissolving 5 g of silver nitrate in triple-distilled water to make a 10 ml solution (solution B). A working solution was prepared by mixing solutions A and B in a 1:2 volume ratio, then poured over the smears and left for 60 minutes at room temperature. The silver colloid formed was washed off with triple-distilled water, and the smears were counterstained with neutral red and safranin 0.5 %. Smears were again washed with triple-distilled water, dehydrated through ascending grades of alcohol, cleared with xylene, and mounted with Dibutylphthalate Polystyrene Xylene (DPX).^[8]

AgNORs are counted as black dots in the nuclei of 100 cells using 100x oil immersion. Mean AgNOR count, size, and

distribution of AgNORs were compared between distinct categories of effusion.^[9]

Distribution of AgNORs in the nuclei were graded as

0 = limited to the nuclei.

1+ = occasional dispersion outside nucleoli.

2+ = Moderate dispersion outside nucleoli.

3+ = widely dispersed throughout the nucleus.

Size variation was graded as

0 = more or less uniform in size.

1+ = two different sizes.

2+ = more than two different sizes.

3+ = all grades and sizes including too minute to be counted.

Statistical Analysis

Sampling Method: A non-probability sampling technique, i.e., a convenience sampling method

Statistical Analysis: All collected data will be compiled and entered a Microsoft Excel worksheet, and analyzed using SPSS 20.0, Jamovi 2.5.6, and MedCalc.

Descriptive statistics for qualitative variables were presented as frequency distributions with percentages, and quantitative variables were presented as means with standard deviations or Medians with interquartile ranges. The tables were represented in suitable diagrammatic and graphical form.

To compare AgNOR counts between the H & E staining sub-groups, the Kruskal-Wallis Test was used, and post-hoc comparisons were performed to identify which two sub-groups differ.

To determine the cut-off value for AgNOR count in the study, ROC analysis is carried out along with Youden's J statistic. Using the cut-off values, the status of AUS and SFM was determined.

The diagnostic evaluation was performed for AgNOR size and dispersion, for ascitic fluid, pleural fluid, and both samples.

RESULTS

The study included 310 patients, of whom 146 were females and 164 were males. Most samples belong to the age groups 41-50 years and 51-60 years, with the average age of the study subjects being 43.1 ± 19.3 years. Of all samples assessed, 176 were ascitic fluid, and 134 were pleural fluid.

Out of 310 samples, 71.95% (223) were Negative for Malignancy, 10.3% (32) were AUS, 5.8% (18) were SFM, and 11.95% (37) were malignant. [Table 1] Out of 146 females, 71.9% were negative for malignancy, 13% were AUS, 4.8% were SFM, and 10.3% were malignant. Out of 164 males, 72% were negative for malignancy, 7.9% were AUS, 6.7% were SFM, and 13.4% were malignant.

Table 1: H & E findings in the study sample

H & E findings	No. of Samples	%
Negative for Malignancy	223	71.95
AUS	32	10.3
SFM	18	5.8
Malignancy	37	11.95
Total	310	100.0

Among the ascitic fluid, 70.5% (124) were Negative for Malignancy, 10.2% (18) were AUS, 5.7% (10) were SFM,

and 13.6% (24) were malignant. Among pleural fluid, 73.9% (99) were Negative for Malignancy, 10.4% (14) were

AUS, 6% (8) were SFM, and 9.7% (13) were Malignancy. [Table 2]

Table 2: H & E findings across types of fluid in the study sample

Type of fluid	H & E n (%)			
	Negative for Malignancy (%)	AUS (%)	SFM (%)	Malignancy (%)
Ascitic fluid	124 (70.5)	18 (10.2)	10 (5.7)	24 (13.6)
Pleural fluid	99 (73.9)	14 (10.4)	8 (6)	13 (9.7)
Total	223 (71.95)	32 (10.3)	18 (5.8)	37 (11.95)

Table 3: Outcome of Samples with agnor count cut-off value of 6.560

Both Ascitic Fluid & Pleural Fluid	Positive n (%)	Negative n (%)
Negative for Malignancy (n=223)	0 (0)	223 (100)
AUS (n=32)	8 (25)	24 (75)
SFM (n=18)	13 (72.2)	5 (27.8)
Malignancy (n=37)	37 (100)	0 (0)
Total (n=310)	58 (18.7)	252 (81.3)

In the study among study samples (both ascitic & pleural fluid), using the agnor count cut-off value of 6.560, the proportions of positivity among the H & E staining are checked. [Table 3]

- Among the Negative for Malignancy, all 223 (100%) were negative.
- Among AUS, 8 (25%) were positive, and 24 (75%) were

negative.

- Among SFM, 13 (72.2%) were positive, and 5 (27.8%) were negative.
- Among malignancies, all 37 (100%) were positive.
- In total, 58 (18.7%) were positive, and 252 (81.3%) were negative.

Table 4: AgNOR dispersion among the study samples

H & E in Total Samples	AgNOR Dispersion n (%)		Total
	0 & 1+	2+ & 3+	
Negative for Malignancy	221 (99.1)	2 (0.9)	223 (71.9)
AUS	22 (68.7)	10 (31.3)	32 (10.3)
SFM	6 (33.3)	12 (66.7)	18 (5.8)
Malignancy	2 (5.4)	35 (94.6)	37 (12)
Total	251 (81)	59 (19)	310 (100)

In the study, among all the samples, using the AgNOR dispersion, the samples were grouped into (0 & 1+) and (2+ & 3+). 251 (81%) were 0 & 1+ and 59 (19%) were 2+ & 3+. [Table 4]

- Among the Negative for Malignancy, 221 (99.1%) were AgNOR dispersion of 0 & 1+, and 2 (0.9%) were AgNOR Dispersion of 2+ & 3+.
- Among the AUS, 22 (68.7%) were AgNOR dispersion

of 0 & 1+, and 10 (31.3%) were AgNOR Dispersion of 2+ & 3+.

- Among the SFM, 6 (33.3%) were AgNOR dispersion of 0 & 1+, and 12 (66.7%) were AgNOR Dispersion of 2+ & 3+.
- Among the Malignancy, 2 (5.4%) were AgNOR dispersion of 0 & 1+, and 35 (94.6%) were AgNOR dispersion of 2+ & 3+.

Table 5: AgNOR size among the study samples

H & E in Total Samples	AgNOR Size n (%)		Total
	0 & 1+	2+ & 3+	
Negative for Malignancy	218 (97.8)	5 (2.2)	223 (71.9)
AUS	22 (68.8)	10 (31.3)	32 (10.3)
SFM	7 (38.9)	11 (61.1)	18 (5.8)
Malignancy	3 (8.1)	34 (91.9)	37 (12)
Total	250 (80.6)	60 (19.4)	310 (100)

In the study, among the study samples (both ascitic & pleural fluid), AgNOR size was used to group into (0 & 1+) and (2+ & 3+). 250 (80.6%) were 0 & 1+ and 60 (19.4%) were 2+ & 3+. [Table 5]

- Among the Negative for Malignancy, 218 (97.8%) were AgNOR size of 0 & 1+, and 5 (2.2%) were AgNOR size of 2+ & 3+.

- Among the AUS, 22 (68.8%) were AgNOR size of 0 & 1+, and 10 (31.3%) were AgNOR size of 2+ & 3+.
- Among the SFM, 7 (38.9%) were AgNOR size of 0 & 1+, and 11 (61.1%) were AgNOR size of 2+ & 3+.
- Among the Malignancy, 3 (8.1%) were AgNOR size of 0 & 1+, and 34 (91.9%) were AgNOR size of 2+ & 3+.

Table 6: Diagnostic evaluation of AgNOR size with H & E among the study subjects

AgNOR Size	H & E n (%)		Total
	Malignancy	Negative for Malignancy	
2+ & 3+	34 (13.1)	5 (1.9)	39 (15)
0 & 1+	3 (1.2)	218 (83.8)	221 (85)
Total	37 (14.2)	223 (85.8)	260 (100)
Diagnostic Evaluation			
Statistic	Value	95% CI	
Sensitivity	91.89%	78.09% to 98.30%	
Specificity	97.76%	94.85% to 99.27%	
Disease Prevalence	14.23%	10.22% to 19.08%	
Positive Predictive Value	87.18%	73.98% to 94.21%	
Negative Predictive Value	98.64%	96.09% to 99.54%	
Accuracy	96.92%	94.03% to 98.66%	

In the study of all samples, among the AgNOR size group (2+ & 3+), 39 were identified, of which 34 were malignant. Five were negative for malignancy by H & E staining, and among the AgNOR size group (0 & 1+), there were 221, of which three were malignancy-positive, and 218 were negative. [Table 6] Malignancy vs. Negative for Malignancy (Excluding AUS,

SFM): Out of 260 samples, 39 (15%) were positive for malignancy (2+ & 3+ AgNOR size), and 221 (85%) were negative for malignancy (0 & 1+ AgNOR size). The AgNOR size test using H & E staining appears to be a promising tool for diagnosing malignancy. It demonstrates high sensitivity, specificity, PPV, NPV, and accuracy.

Table 7: Diagnostic evaluation of AgNOR dispersion with H & E among the study subjects

AgNOR Dispersion	H & E n (%)		Total
	Malignancy	Negative for Malignancy	
2+ & 3+	35 (13.5)	2 (0.75)	37 (14.2)
0 & 1+	2 (0.75)	221 (85)	223 (85.8)
Total	37 (14.2)	223 (85.8)	260 (100)
Diagnostic Evaluation			
Statistic	Value	95% CI	
Sensitivity	94.59%	81.81% to 99.34%	
Specificity	99.10%	96.80% to 99.89%	
Disease Prevalence	14.23%	10.22% to 19.08%	
Positive Predictive Value	94.59%	81.46% to 98.59%	
Negative Predictive Value	99.10%	96.63% to 99.77%	
Accuracy	98.46%	96.11% to 99.58%	

In the study samples, among the AgNOR dispersion group (2+ & 3+), 37 were identified, of which 35 were malignant. Two were negative for malignancy by H & E staining, and among the AgNOR size group (0 & 1+), 223 were malignancy-positive, of which two were malignancy-negative, and 221 were malignancy-negative. [Table 7] Malignancy vs. Negative for Malignancy: Out of 260 samples, 37 (14.2%) were positive for malignancy (2+ & 3+ AgNOR size), and 223 (85.8%) were negative for malignancy (0 & 1+ AgNOR size). Indicating AgNOR dispersion in H&E staining appears to be a highly accurate tool for diagnosing malignancy. It demonstrates excellent sensitivity, specificity, PPV, NPV, and overall accuracy. These results suggest that the test is reliable in both identifying malignant cases and ruling out malignancy.

DISCUSSION

Serous effusions may occur due to a variety of etiologies, including nonmalignant and malignant effusions. In most cases, the cellular response is nonspecific and produces a variety of cells, including mesothelial cells, macrophages, erythrocytes, neutrophils, lymphocytes, and other leucocytes. The key role of cytopathology is to detect malignant cells in these effusions.^[10,11] On cytological

evaluation of these effusions, it often results in atypical cells that are difficult to differentiate from benign or malignant cells. To overcome this, AgNOR staining can be used to distinguish atypical cells as benign or malignant, as AgNOR count is significantly elevated in malignant cells.^[12] Nucleolar organizing regions are loops of DNA that can be visualized using the AgNOR staining technique. Many studies have examined the utility of AgNOR staining for differentiating benign and malignant cells across various body tissues.^[13,14] But studies were limited in their assessment of the utility of AgNOR staining in serous effusions. The present study was conducted to differentiate benign from malignant effusions using AgNOR staining, particularly in effusions with atypical cells that cannot be differentiated morphologically. The present study is a cross-sectional study conducted in the Department of Pathology, Basaveshwara Medical College, and Hospital, Chitradurga, between August 2022 and December 2023. The present study includes 310 patients with effusion, the largest of the studies compared. Of 310 patients, 146 were females, and 164 were males, with a male-to-female ratio of 0.8:1, comparable to studies by Junwal A et al,^[15] and Karki S et al.^[3] But M: F ratio of 4.33:1, which is more in the study conducted by Palathingal D M R et al.^[16] The mean age of the study population is 43.1 ± 19.3, which is

comparable to the studies conducted by Junwal A et al,^[15] and Karki S et al.^[3]

This study included 310 patients: 134 with pleural effusion and 176 with peritoneal fluid. A survey conducted by Junwal A et al,^[15] contains 39 pleural, 55 ascitic fluid, and three pericardial effusions. A study conducted by Karki S et al,^[3] included 71 pleural and 103 ascitic fluids.

On H and E staining: out of 310 cases, 223 were negative

for malignancy, 32 were Atypia of undetermined significance. Eighteen were suspicious of malignant, and 37 were malignant. Similarly, a study conducted by Karki S et al,^[3] included 132 benign, 10 atypical, and 32 malignant effusions. A survey conducted by Gill M et al,^[8] included 57 benign, 15 atypical, and 28 malignant effusions. Sujathan K et al,^[17] had 37 benign, eight atypical, and 55 malignant effusions. Palathingal D M R et al,^[16] had 69 benign and 11 malignant effusions. [Table 8]

Table 8: Distribution of effusions based on H and E stain in different studies

	Karki S et al [3]	Gill M et al [8]	Sujathan K et al [17]	Palathingal D M R et al [16]	Present study
NFM(Benign)	132	57	37	69	223
AUS	10	15	8	-	32
SFM					18
Malignant	32	28	55	11	37

AgNOR count: In the present study, the mean AgNOR count of NFM/benign effusion is 2.33+/-0.67, AUS is 5.68+/-1.99, SFM is 7.87+/-2.25, and Malignant is 11.04+/-0.9 with a significant p value of 0.000. These values are comparable to

those reported in studies by Karki S et al.^[3] But lower mean AgNOR count is seen in studies conducted by Gill M et al,^[8] Sujathan K et al,^[17] and Palathingal D M R et al.^[16] [Table 9]

Table 9: Comparison of mean AgNOR count in different studies

	Karki S et al [3]	Gill M et al [8]	Sujathan K et al [17]	Palathingal D M R et al [16]	Present study
NFM	2.12+/-0.54 (1.23-3.88)	1.53+/-0.15 (1.29-1.86)	1.92+/-0.23 (1.51-2.42)	1.14+/-0.07 (0.92-2.93)	2.33+/-0.67 (1.27-3.76)
AUS	8.77+/-2.97 (2.04-10.09)	3.99+/-0.59 (2.78-3.84)	3.74+/-1.50 (1.92-5.53)	-	5.68+/-1.99 (2.64-10.32)
SFM					7.87+/-2.25 (3.47-10.63)
Malignant	10.43+/-0.73 (9.55-12.02)	4.03+/-0.38 (2.78-50.1)	4.72+/-0.76 (3.13-6.9)	2.23+/-0.22 (3.21-6.82)	11.04+/-0.9 (9.36-12.77)
P value	-	0.0001	-	<0.001	0.000

Using the mean AgNOR count cutoff of 6.560, all 100% of NFM were below the cutoff. 75% of AUS were below the cutoff, and 25 % of AUS were above the cutoff. 27.8% of SFM were below the cut, and 72.2% of SFM were above the cut-off. 100% of malignancies were above the cut-off. Clearly indicating that the mean AgNOR count of benign cells was below the cut-off, and the mean AgNOR count of malignant cells was above the cut-off. Thus, the cutoff of the mean AgNOR count on ROC helps differentiate atypical cells (AUS and SFM) from benign and malignant cells, thereby overcoming the disadvantage of H and E stain.

AgNOR dispersion: It was classified into 0.1+, 2+, and 3+ based on dispersion, limited to nucleoli or away from them, according to criteria established by Ahsan et al. In the present study, 99.1% of negative for malignancy on H and E belong to the group 0 and 1+, whereas 94.6% of malignancy on H

and E belong to the groups 2+ and 3+, clearly indicating less dispersion in benign cells and greater dispersion in malignant cells. Similar observations were noted in studies conducted by Junwal A et al,^[15] and Karki S et al,^[3] with a statistically significant p-value of <0.0001.

AgNOR size: It was classified into 0.1+, 2+, and 3+ based on AgNOR dot size. In the present study, 97.8% of negative for malignancy on H and E belong to the zero and 1+ groups, whereas 91.9% of malignancy on H and E belong to the 2+ and 3+ groups, clearly demonstrating uniform size in benign cells and variable size in malignant cells. Similar observations were noted in studies conducted by Junwal A et al,^[15] and Karki S et al,^[3] with a statistically significant p-value of <0.0001.

[Table 10]

Table 10: Comparison of diagnostic parameters of AgNOR size and AgNOR dispersion in differentiating benign and malignant

		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)
AgNOR size	Junwal A et al [15]	100	84.1	39.2	100	85.6
	Present study	91.89	97.76	87.18	98.64	96.92
AgNOR dispersion	Junwal A et al [15]	100	79.5	33.3	100	81.4
	Present study	94.59	99.10	94.59	99.10	98.46

On evaluation, AgNOR size had a sensitivity of 91.89%, a specificity of 97.76%, a PPV of 87.18% and an NPV of 98.64%. Sensitivity, specificity, and NPV were comparable, but PPV was lower in the study conducted by Junwal A et al.^[15]

AgNOR dispersion had a sensitivity of 94.59%, a Specificity of 99.10%, a PPV of 94.59% and an NPV of 99.10%; all these values are comparable except that PPV is lower in the study conducted by Junwal A et al.^[15]

CONCLUSION

The mean AgNOR count is significantly elevated in malignant cells compared with benign cells. Along with increased size and dispersion, an AgNOR count is seen in malignant cells of serous effusion. Hence, AgNOR staining helps in differentiating atypical cells on routine H and E staining into benign and malignant cells. Therefore, the AgNOR stain is an extremely useful and rapid diagnostic test in differentiating benign and malignant cells where routine cytology fails. Further studies with a larger sample size are required to conclude this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Takahashi M. Effusions in body cavities. In: Takahashi M, editor, Color atlas of cancer cytology, 2nd ed, New York: George Thieme Verlag Stuttgart; 1981.p. 427.
2. Tiniakos DG, Healicon RM, Hair T, Wadehra V, Horne CH, Angus B. P53 immunostaining as a marker of malignancy in cytologic preparation of body fluids. *Acta Cytol* 1995; 39(2):171-6.
3. Karki S, Jha A, Sayami G. The role of argyrophilic nucleolar organizer region (AgNOR) study in cytological evaluation of fluids, especially for detection of malignancy. *Kathmandu University Medical Journal* 2012;10(1):34-39. Available from: <http://dx.doi.org/10.3126/kumj.v10i1.6913>.
4. Mezger J, Stotzer O, Schilli G, Bauer S, Wilmanns W. Identification of carcinoma cells in ascitic and pleural fluid: Comparison of four panepithelial antigens with carcinoembryonic antigen. *Acta Cytol* 1992;36:75-81.
5. Ferguson-Smith MA, Ilandmaker SD. Observations on the satellite human chromosomes. *Lancet* 1961;1:638-40.
6. Rocher A, Blanco AM, Palaoro L. Usefulness of AgNOR assay in the assessment of serous effusions. *Sociedad Medica de Santiago* 2000; 128(9):963-6812.
7. Pinto D, Chandra A, Crothers BA, Kurtycz DFI, Schmitt F. The international system for reporting serous fluid cytopathology-diagnostic categories and clinical management. *J Am Soc Cytopathol* 2020;9(6):469-477 Available from: <http://dx.doi.org/10.1016/j.jasc.2020.05.015>.
8. Gill M, Singh U, Mahapatra QS, Gehlot S, Gupta V, Sen R. Role of argyrophilic nucleolar organizer region staining in identification of malignant cells in effusion. *J Cytol [Internet]*. 2011;28(4):191-5. Available from: <http://dx.doi.org/10.4103/0970-9371.86346>.
9. Ahsan S, Tayyab M, Chaudhry NA, Khan SA. Silver staining nucleolar organizer region (AgNOR) typing in nodular hyperplasia of the prostate. *Pak Postgraduate Med J*. 1992;2(3):67-72.
10. Murphy WM, Ng ABP. Determination of primary site by examination of cancer cells in body fluids. *American Journal of Clinical Pathology* 1972;58(5):479-88. Available from: <http://dx.doi.org/10.1093/ajcp/58.5.479>.
11. Spriggs AI, Boddington MM. *The cytology of effusions*. New York: Grune & Stratton Inc 1968.
12. Chai SM, Van Vliet C. Cytological diagnosis of malignant pleural mesothelioma. *Curr Pulmonol Rep [Internet]*. 2017;6(1):1-8. Available from: <http://dx.doi.org/10.1007/s13665-017-0159-y>.
13. Allen JP, Gallimore AP. Nucleolar organizer regions in benign and malignant glandular lesions of the cervix. *J Pathol*. 1989; 158: 41-44.
14. Egan MJ, Crocker J. Evaluation of nucleolar organizer regions in pulmonary pathology. *Thorax* 1990; 45: 225-32.
15. Junwal A, Malik R, Balani S, Meena RK, Jathapi S. Role of argyrophilic nucleolar organizer region (AgNOR) study in cytological evaluation of serous fluids for detection of malignancy. *J evol med dent sci [Internet]*. 2020;9(46):3469-73. Available from: <http://dx.doi.org/10.14260/jemds/2020/759>.
16. Palathingal DMR, Dept of Pathology, Government Medical College, Thrissur, Kerala. Role of argyrophilic nucleolar organiser region staining in effusions for detection of malignancy - diagnostic test evaluation. *J Med Sci Clin Res [Internet]*. 2017;5(9). Available from: <http://dx.doi.org/10.18535/jmscr/v5i9.71>.
17. Sujathan K, Kannan S, Pillai KR, Chandralekha B, Amma NS, Nair MK. Significance of AgNOR count in differentiating malignant cells from reactive mesothelial cells in serous effusions. *Acta Cytol [Internet]*. 1996;40(4):724-8. Available from: <http://dx.doi.org/10.1159/000333946>.