RESULTS

This study was carried out on two groups:

Group I: 50 patients suffering from conjunctivitis.

Group II: 20 individuals with ophthalmologic complaint rather than conjunctivitis.

Table (1) shows incidence of Adenovirus infection among studied groups using immunofluorescent staining of the direct swabs:

- 45 cases (90%) were positive for the presence of adenovirus antigens in their specimens, while 5 cases (5%) were negative in group I and only 4 (20%) were positive, while 16 (80%) were negative in group II.
- The previous results are shown in Fig (1)

Incidence of Adenovirus infection among studied groups using cell culture of the swabs are shown in **table (2):**

- 30 cases (60%) were positive for adenovirus CPE in their specimens, while 20 cases (40%) were negative in group I. All cases were negative for the presence of adenovirus CPE in their specimens in group II.
- These results are demonstrated in Fig (2).

Table (3) shows incidence of Adenovirus infection between studied groups using immunofluorescent staining after cell culture of the swabs: 33 cases (66%) were positive for adenovirus antigens, while 17 cases (34%) were negative in group I.

These 33 positive cases were classified as follow:

- The 30 cases which were positive for cell culture were strong positive (++++) by immunofluorescent staining, while 3 cases among those which were negative for cell culture were moderately positive (++) by immunofluorescent staining.
- Among group II, 18 cases (90%) were negative for cell culture and immunofluorescent staining, while only 2 cases (10%) were negative for cell culture and by immunofluorescent staining they were weakly positive (+).

Fig (3) shows incidence of Adenovirus infection between studied groups using immunofluorescent staining after cell culture of the swabs.

Table (4) shows Comparison between results of cell culture, immunofluorescent staining of direct of direct swabs and immunofluorescent staining after cell culture in group I:

- 45, 30 and 33 cases were positive by using immunofluorescent staining of direct swabs, cell culture and immunofluorescent staining after cell culture respectively.
- 5, 20 and 17 cases were negative by using immunofluorescent staining of direct swabs, cell culture and immunofluorescent staining after cell culture respectively.

Table (5) shows Comparison between results of cell culture, immunofluorescent staining of direct of direct swabs and immunofluorescent staining after cell culture in group II:

- 4 cases were positive by using direct immunofluorescent staining, 2 cases were positive by using immunofluorescent staining after cell culture, while no cases were positive for cell culture.
- 16 cases were negative by using immunofluorescent staining of direct swab.

- 18 cases were negative by using immunofluorescent staining after cell culture, while all the 20 cases were negative for cell culture.

Table (6) shows accuracy of immunofluorescent staining of direct swabs in relation to cell culture technique.

- 26 cases were positive by both tests (true positive), while one case were negative by both tests (true negative).
- The sensitivity of immunofluorescent staining of direct swab was 86.7% and the accuracy was 54% while the specificity was 5%.
- Positive predictive value was 57.7%, while negative predictive value was 20%.

Table (7) shows accuracy of immunofluorescent staining after cell culture in relation to cell culture technique.

- 30 cases were positive by both tests (true positive), while 17 cases were negative by both tests (true negative).
- The sensitivity of immunofluorescent staining after cell culture was 100%, and accuracy was 94% and the specificity was 85%.
- Positive predictive value was 90.9% while negative predictive value was 9.1%.

Table (1): Incidence of Adenovirus infection among studied groups using immunofluorescent staining of the direct swabs

Groups / Adenovirus	Group (I) Cases		Group (II) Control	
	Number	(%)	Number	(%)
Positive	45	90	4	20
Negative	5	10	16	80
Total	50	100	20	100

$\chi^2 = 46.1$ $P < 0.001$

Positive results were significantly higher in group I than in group II.

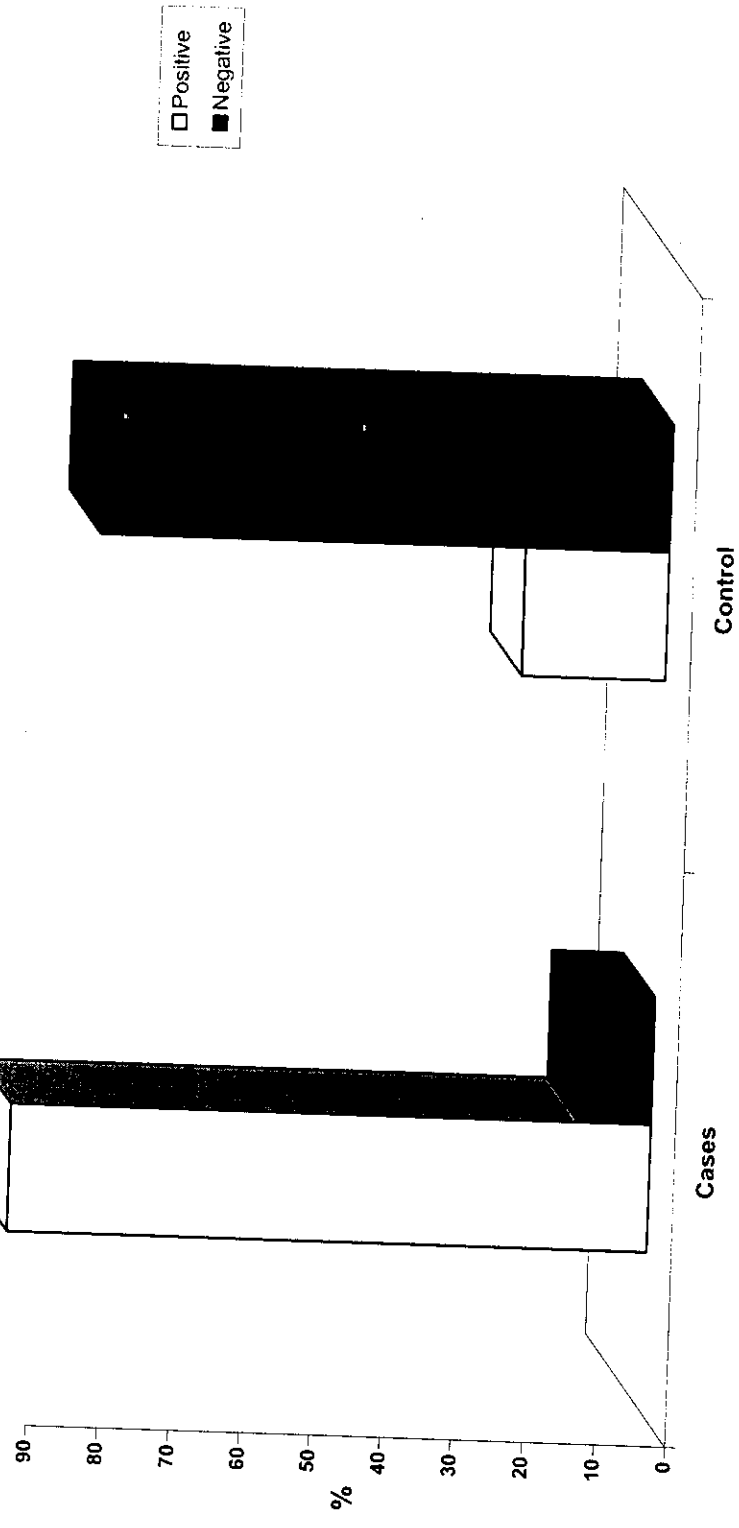

Fig (1): Incidence of adenovirus infection among studied groups using immunofluorescent staining of direct swabs.

Table (2): Incidence of Adenovirus infection among studied groups using cell culture of the swabs.

Groups / Adenovirus	Group (I) Cases		Group (II) Control	
	Number	(%)	Number	(%)
Positive	30	60	0	0
Negative	20	40	20	100
Total	50	100	20	100

$\chi^2 = 21.5$ $P<0.001$

Positive results were significantly higher in group I than in group II.

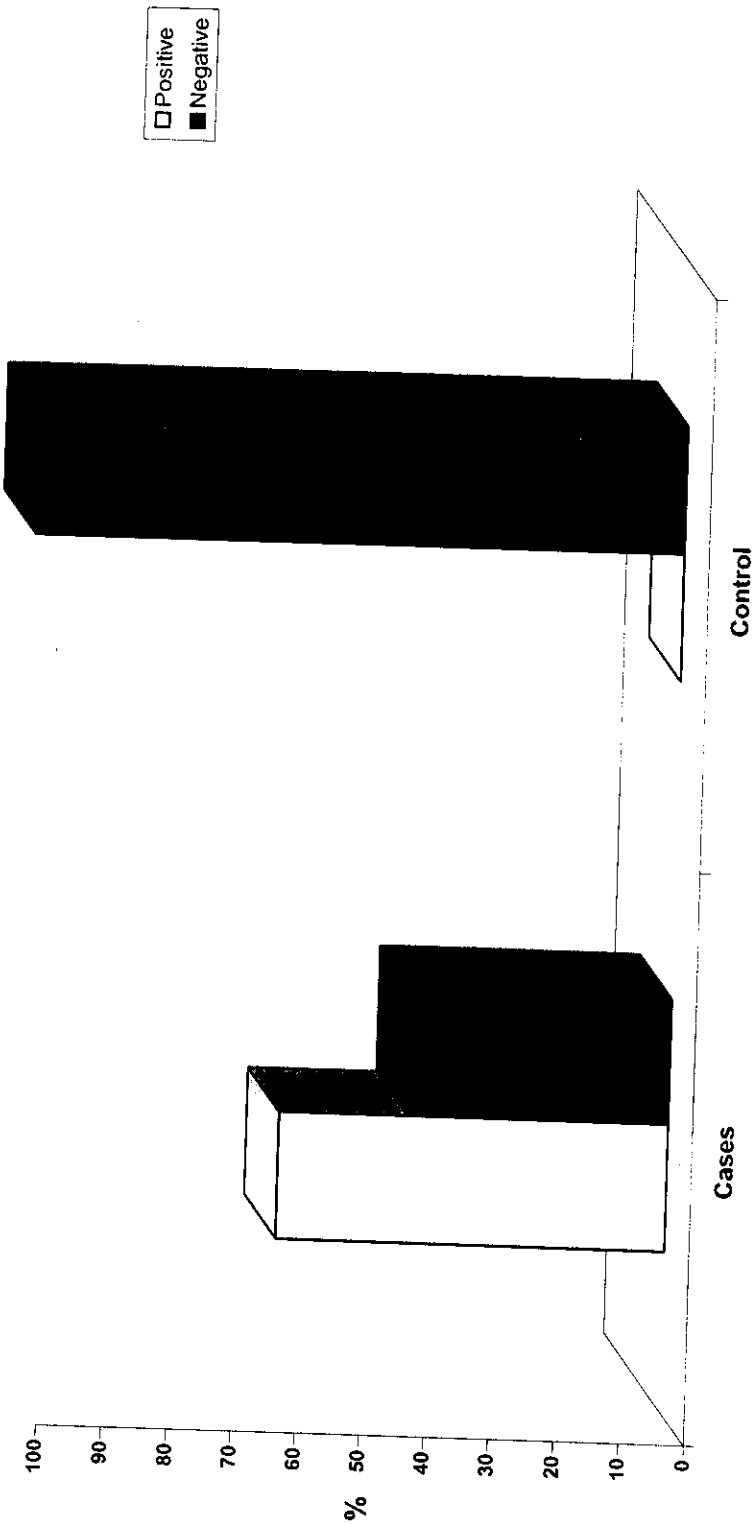

Fig (2): Incidence of adenovirus infection among studied groups using cell culture of the swabs.

Table (3): Incidence of Adenovirus infection between studied groups using immunofluorescent staining after cell culture of the swabs.

Groups / Adenovirus	Group (I) Cases		Group (II) Control	
	Number	(%)	Number	(%)
Positive by IF	33	66	2	10
Positive for cell culture	30	60	0	0
Negative for cell culture	3	6	2	10
Negative by IF	17	34	18	90
Positive for cell culture	0	0	0	0
Negative for cell culture	17	34	18	90
Total	50	100	20	100

$\chi^2 = 22.4$ $P<0.001$

Positive results were significantly higher in group I than in group II.

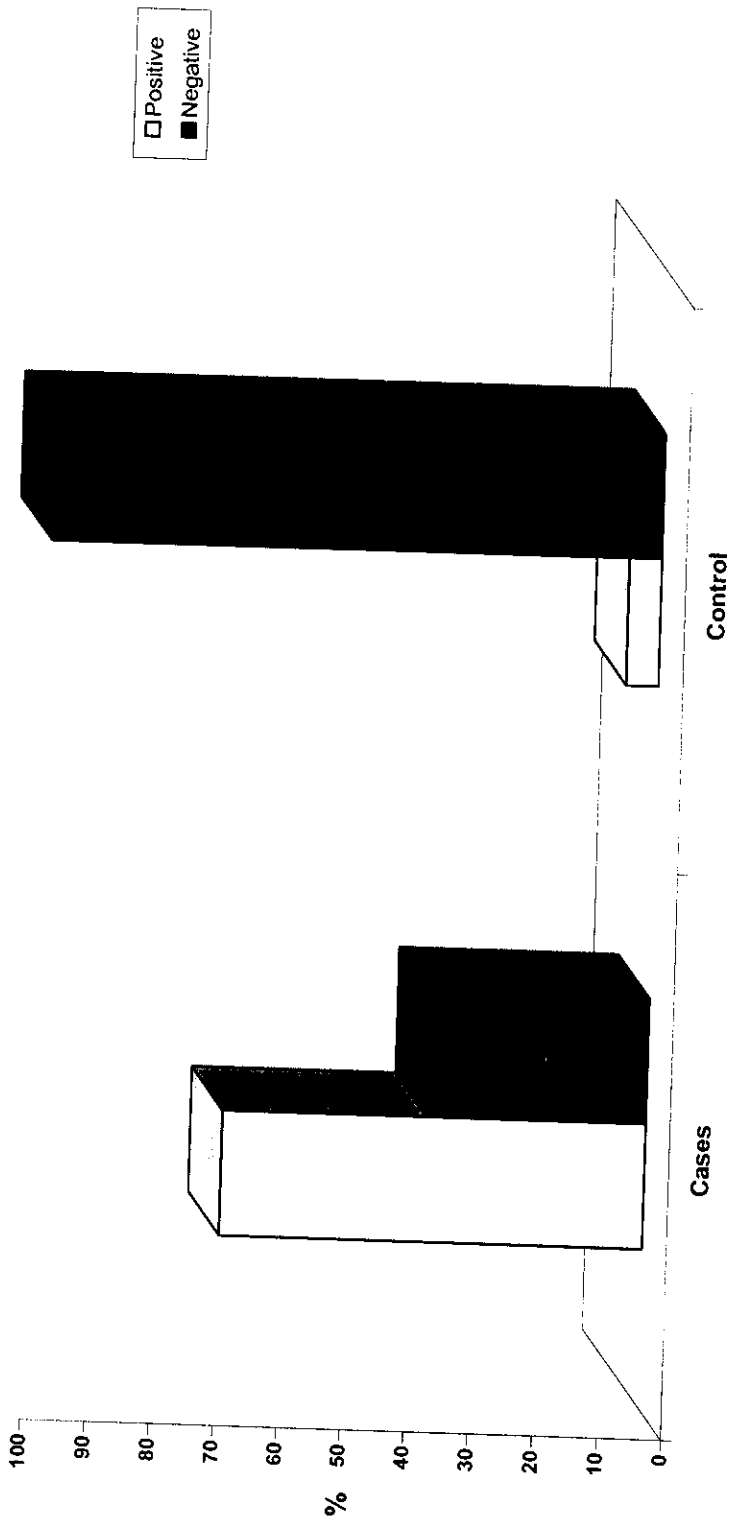

Fig (3): Incidence of adenovirus infection among studied groups using immunofluorescent after cell culture of the swabs.

Table (4): Comparison between results of immunofluorescent staining of direct swabs, cell culture and immunofluorescent staining after cell culture in group I.

	Immunofluorescent staining of direct swabs		**Cell Culture**		**Immunofluorescent staining after cell culture**	
	Number	(%)	Number	(%)	Number	(%)
Positive	45	90	30	60	33	66
Negative	5	10	20	40	17	34
Total	50	100	50	100	50	100

Table (5): Comparison between results of immunofluorescent staining of direct swabs, cell culture and immunofluorescent staining after cell culture in group II.

	Immunofluorescent staining of direct swabs		**Cell Culture**		**Immunofluorescent staining after cell culture**	
	Number	(%)	Number	(%)	Number	(%)
Positive	4	20	0	0	2	10
Negative	16	80	20	100	18	90
Total	20	100	20	100	20	100

Table (6): Accuracy of immunofluorescent staining of direct swabs in relation to cell culture technique.

Immunofluorescent staining of direct swabs	**Cell culture**		**Total**
	Positive	**Negative**	
	N (%)	N (%)	N (%)
Positive	26 (86.7%) True positive	19 (95%) False positive	45 (90%)
Negative	4 (13.3%) False negative	1 (5%) True negative	5 (10%)
Total	30 (100%)	20 (100%)	50 (100%)

Sensitivity = 86.7%

Specificity = 5%

Accuracy = 54%

Positive predict value (PPV) = 57.7%

Negative predict value (NPV) = 20%

% false +ve =42.2%

% false -ve =80%

Table (7): Accuracy of immunofluorescent staining after cell culture in relation to cell culture technique.

IF after cell culture	Cell culture		Total
	Positive	Negative	
	N (%)	N (%)	N (%)
Positive	30 (66%) True positive	3 (6%) False positive	33 (66%)
Negative	0 (0%) False negative	17 (34%) True negative	17 (34%)
Total	30 (100%)	20 (100%)	50 (100%)

Sensitivity = 100%

Specificity = 85%

Accuracy = 94%

Positive predict value (PPV) = 90.9%

Negative predict value (NPV) = 100%

% false +ve = 9.1%

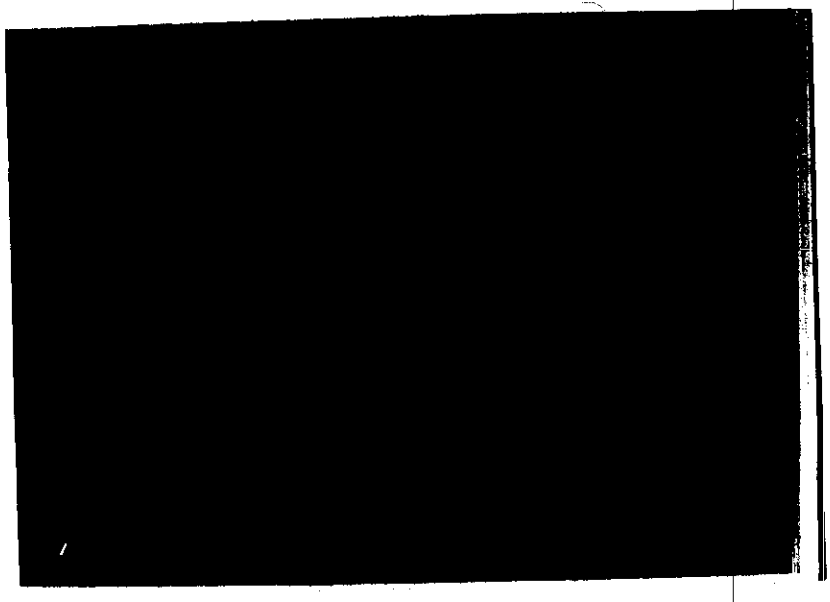

Fig (4): Complete monolayer sheet of HEP-2 cells

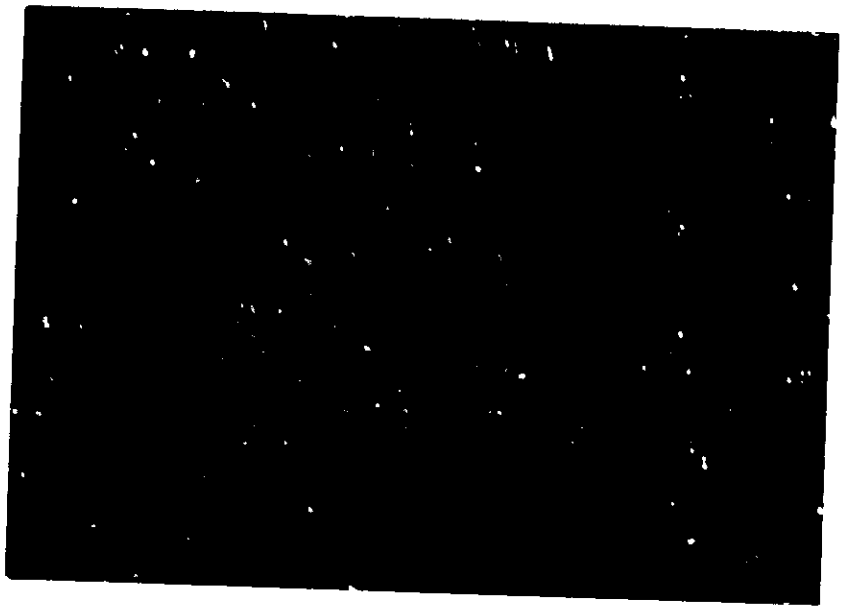

Fig (5): Cytopathic effect (CPE) of adenovirus onto HEP-2 cells

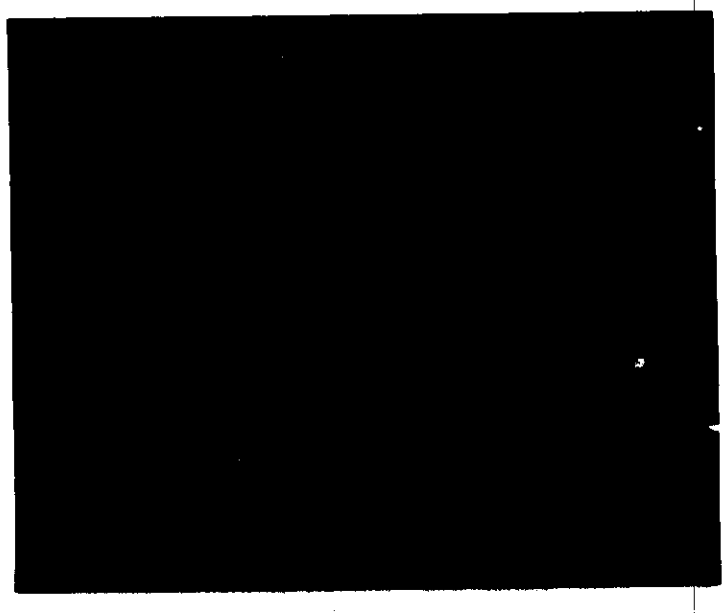

Fig (6): Immunofluorescent staining of HEP-2 cells

DISCUSSION

The aim of the present study was to evaluate the incidence of adenovirus keratoconjunctivitis by using immunofluorescent staining of direct swabs, cell culture and immunofluorescent staining after cell culture to compare between the results.

The immunofluorescent staining of direct swabs can only be recommended if a small number of specimens are submitted and examined by skilled and experienced microscopist (Elfath, et al 1999).

As regards to immunofluorescent staining of direct swabs results showed that in group I, out of 50 patients diagnosed clinically as having viral conjunctivitis, 45 cases (90%) were positive for adenovirus antigens and 5 cases (10%) were negative. Similar results were detected by Ursula, et al (2002) where immunofluorescent staining of direct swabs results was positive in 14 cases (92.8%) out of 15 cases were positive for adenovirus by using immunofluorescent staining of direct swabs.

Out of 20 control cases (group II), 16 cases (80%) were negative for adenovirus antigens, while 4 cases (20%) were positive and considered as false positive.

These false positive results explained by Ursula et al. (2002) as they mentioned that, the immunofluorescent staining of direct swabs is less sensitive than tissue culture because immunofluorescent staining of direct swabs produce false positive results due to cross-reactivity with contaminating staphylococcal protein A.

Tissue culture is considered to be the gold Standard method for the identification of adenovirus (Adbikary et al., 2001).

By using cell culture technique in group I 30 cases (60%) were positive for adenovirus and 20 cases (40%) were negative. These results agreed with that obtained by (Elfath, et al 1999). Who found that out of 415 eye swabs, 248 (59%) were positive by cell culture isolation and disagreed with (Eiilchi et al., 2000) who reported that out of 478 cases, 84 cases (80.3%) were diagnosed as having adenoviral conjunctivitis by cell culture method and disagreed with (Peter et al, 1982) as they isolated adenovirus from 11(8%) cases of conjunctivitis out of 140 cases. This disagreement is may be due to the difference between the number of cases in both studies.

By using immunofluorescent staining after cell culture technique 33 cases (66%) in group I were positive for adenovirus antigens, from which 30 cases (60%) were strong positive by immunofluorescent staining and positive for cell culture. While 3 cases (6%) were moderately positive by immunofluorescent staining after cell culture and negative for cell culture, these three cases were considered as false positive results. This partially agreed with John et al. (1993) who reported that, 18 cases (69.2%) out of 26 cases were positive for adenovirus antigens by using immunofluorescent staining after cell culture.

In group II, inspite of all cases were negative for cell culture, 2 cases only (10%) were weakly positive by immunofluorescent staining after cell culture and considered as false positive results while 18 cases (90%) were negative by both cell culture and immunofluorescent staining after cell culture, these false positive results may be due to increase the sensitivity of fluorescent dye.