

Performance of the *UroVysion*[®] FISH assay for the diagnosis of malignant effusions using two cutoff strategies

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Disclosure Information

I hereby declare that I have had business or personal interests in the following industrial enterprises since 1 September 2017:

Name of the enterprise / Nature of the interest

Enterprise | Interest

None

Background

- Malignancy is a common cause of effusion
- Most of cancer patients can not tolerate invasive procedures
- Fluid cytology provides diagnosis in about 60% of cases, in a short turn-around-time (TAT)
- Under special conditions, especially in cases of suspicious cytology, the association of complementary methods is of great interest in clinical laboratories

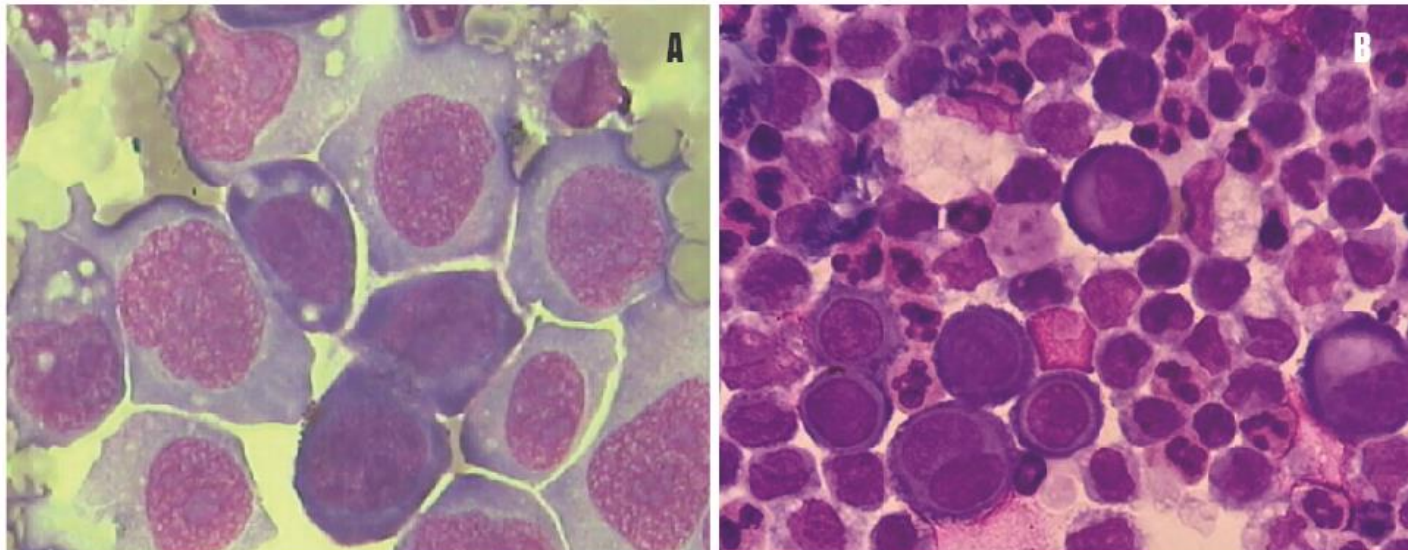
Objectives

- ❖ To evaluate the detection of aneuploid cells in samples of pleural and peritoneal fluids with the fluorescence *in situ* hybridization (FISH) assay – *UroVysion*[®] using two different strategies of cutoff

Methods

- **Cytological examination**

- Nucleated cells were counted in a counting (Neubauer) chamber, and the fluid samples were centrifuged (2000 *rpm*, 10 *minutes*) to prepare the slides;
- Cytological examination (cell differential and oncotic cytology) was performed on slides stained with hematological dye (Leishman)



Legend: A. Tumor cells clustering in case of malignant pleural effusion (*Leishman*); B. Reactive mesothelial clustering of cells in benign pleural effusion (*Leishman*).

Methods

Seventy samples of pleural or peritoneal fluid with positive (n=40), negative (n=15) or suspicious (n=15) cytology were evaluated by FISH assay (*UroVysion*[®]) with probes that bind to the centromeric region of the chromosomes **3** (red-labeled), **7** (green-labeled), **17** (blue-labeled) and the **9p21** region (yellow-labeled), respectively. The manufacturer's instructions were followed with slight modifications to time and temperature.

Methods

- **Proposed cutoff (P-FISH)**

Inverse β function, where:

$\alpha = 1 + X$ (X represents the highest number of positive signals obtained by the observers) and $\beta =$ number of cells analyzed

Analyses: **200** interphase cells/sample

Aneuploidy if:

Chromosome 3: > 3.0% for one signal or > 3.0% for 3 or more signals;

Chromosome 7: > 4.0% for one signal or > 2.0% for 3 or more signals;

Chromosome 17: > 4.0% for one signal or > 3.0% for 3 or more signals;

Chromosome 9p21: > 4.6% for one signal

- **Manufacturer's cutoff (M-FISH)**

Analyses: **25** interphase cells/sample

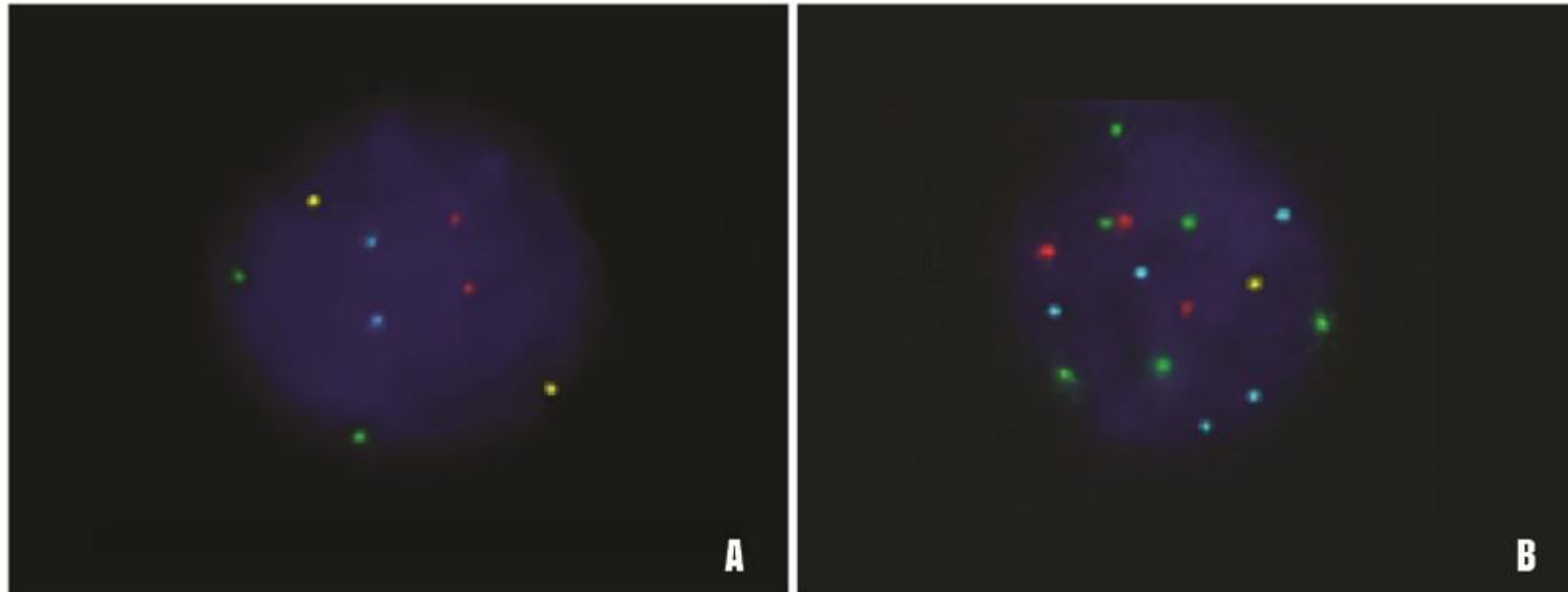
Aneuploidy if:

Cell presenting ≥ 4 gains for 2 or more chromosomes (3, 7 or 17)

or ≥ 12 cells with zero signal for 9p21.

Methods

- Example of an euploid cell in a benign effusion and an aneuploidy cell in a case of malignant pleural effusion.



Legend: **A.** Pleural effusion cells showing euploid cells (2n) for chromosomes 3 (red), 7 (green), 17 (blue) and 9p21 (yellow); FISH, UroVysion, 1000x; **B.** Pleural effusion cells showing aneuploidy for chromosomes 3 (red), 7 (green), 17 (blue) and 9p21 (yellow); FISH, UroVysion, 1000x.

Results

- FISH performance was evaluated using two different cutoffs: the manufacturer's cutoff (*M-FISH*) and a proposed cutoff (*P-FISH*)

	S (%)	E (%)	PPV (%)	NPV (%)	ACU (%)
Cytology	85.7	85.7	98.1	40.0	85.7
<i>M-FISH</i>	57.1	85.7	97.2	18.1	60.0
<i>P-FISH</i>	87.3	71.4	96.4	38.4	85.7
Cytology and <i>M-FISH</i>	81.3	83.3	97.2	38.4	81.6
Cytology and <i>P-FISH</i>	88.0	83.3	98.1	41.0	87.8

S: Sensitivity; E: Specificity; PPV: positive predictive value; NPV: negative predictive value; ACU: accuracy.

Results

Comparative analysis between cytology and *UroVysion*[®] *FISH*

Test	<i>p</i> *
Cytology vs. <i>M-FISH</i>	< 0.0006
Cytology vs. <i>P-FISH</i>	NS
<i>M-FISH</i> vs. <i>P-FISH</i>	< 0.0006

* $p < 0.05$ significant

NS: Not Significant

Conclusion

- The present study showed that *UroVysion*[®] *P-FISH* was highly effective to identify aneuploid cells in fluid samples from patients with malignant effusions;
- *UroVysion*[®] *P-FISH* exhibited good sensitivity and accuracy mainly in cases of inconclusive cytology;
- The results are promising, but its clinical application still demands the replication of this procedure in samples from a wider spectrum of malignancies.