ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2023; 28(2): 3893-3899
doi: 10.25083/rbl/28.2/3893.3899

**Received for publication, September, 06, 2023**
**Accepted, September, 21, 2023**

*Original article*

# The role of lactate dehydrogenase of the pleural liquid in the cytopathological diagnosis

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## Abstract

Elevated serum or pleural fluid lactate dehydrogenase (LDH)enzyme levels can be associated with various conditions, including neoplasia, reticuloendothelial tumors, and leukemia. These conditions can lead to cell damage or death, causing LDH to be released into the bloodstream and pleural fluid. An increase in LDH levels may prompt further investigation into the underlying cause, which could include malignancies or other diseases. Elevated LDH levels in pleural fluid are often associated with certain conditions, and our study seeks to establish a quantitative assessment of LDH in conjunction with cytopathological examination for diagnostic purposes.

We show here that the presence of abnormal or malignant cells in pleural fluid, as indicated by positive cytopathological results, can be concerning and often necessitates further evaluation. Elevated LDH levels provide biochemical evidence that supports the cytopathological findings, strengthening the case for a potential underlying disease or condition.

## Keywords

LDH, citology, pleural liquid

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## Introduction

Cytology is a diagnosis method in malignant diseases and the most important method for early detection of neoplasms with different locations. Exfoliative cytology was the first method with practical application in the diagnosis of malignant tumours of the cervix. Due to the accuracy of this cytodiagnostic method, it has also been applied in the diagnosis of lung tumours.

Comparing the cytological results with the histological results, it was found that there was 80-90% concordance between the examinations. In order to obtain good quality cytological results, the cytologist needs to receive information on the patient's clinical and imaging datasets.

There are multiple major arguments supporting the importance of cytological diagnosis:

Cytology is a quick and inexpensive method compared to other diagnostic methods. The low psychological impact of cytology is important for the patient, as the waiting period for diagnosis is very short. Cytology helps in guiding therapy in inoperable patients. Cytology is the method that can identify the cell type in lung carcinoma, thus avoiding biopsy. In some cases, cytology is the only possible method of diagnosis. This method targets patients who refuse biopsy and cases where the puncture fragments are very small or cases where malignant intraepithelial lesions have no endoscopic expression.

The cytological examination is the procedure by which cells can be examined to identify their atypical features. Fine needle aspiration cytology (FNA) of serous membrane effusions can play an important role in the diagnostic analysis of both primary and metastatic disease. From this perspective, liquid-based cytology (LBC) represents a feasible and reliable method to empower the performance of ancillary techniques (i.e. immunocytochemistry and molecular testing) with high diagnostic accuracy [1].

The cell is plastic and sensitive to intracellular and extracellular changes. Through its components, it can adapt to variations, maintaining internal homeostasis, and if these variations exceed normal physiological values, cell abnormalities occur.

The cell membrane, the cytoplasm with its organelles and the nucleus and the coordinator of cellular activities play an active role in the cell's adaptation.

On smears, the cell membrane rarely appears as a structure different from the cytoplasm. In cytological examination it is particularly important to examine the cell boundaries as only morphologically intact cells will be interpreted. The sampling and smear methods play an important role in avoiding cellular artefacts.Cells with unclear membrane boundaries can induce diagnostic confusion by overestimating the ratio of nucleus to cytoplasm.

The cytoplasm is made up of the cytosol, which includes the cell organelles.The cell nucleus consists of chromatin, nucleoli and nucleoplasm, surrounded by the two sheets of the nucleolus. The nucleus is in an interdependent relationship with the cytoplasm, the existence of one being strictly conditional on the existence of the other. The nucleus is the holder of the genetic code and consists of nucleic acids (DNA, RNA) and proteins.

The following main aspects are taken into account when formulating the cytological diagnosis: the location of the nucleus , the shape of the nuclear membrane, the nucleocytoplasmic ratio

, the type and distribution of chromatin (finely granular or in large blocks), the presence, number and size of nucleoli, the presence and type of mitoses.

## Normal exfoliative cytology of pleural fluids

The non-reactive mesothelial cell has variable shapes with large dimensions. The nucleus of the mesothelial cell may be single or multiple, being euchromatic with an obvious nucleolus or heterochromatic with an irregular outline. In most non-reactive mesothelial cells the cytoplasm is rich, so that the nucleus-to-cytoplasm ratio is subunitary as shown in Figure 1.

Other cell types found in pleural fluid are: erythrocytes, lymphocytes, polymorphonuclear, eosinophils, etc. and reactive mesothelial cells in pleural fluids.In pleural fluids, reactive mesothelial cells show discrete morphological changes compared to non-reactive mesothelial cells.

Increased RNA is reflected by more intense basophilia of the cytoplasm, as well as the presence of multiple large nucleoli and pleomorphism.

Reactive mesothelial cells show an increased nucleocytoplasmic ratio and adhere to each other in small clusters.

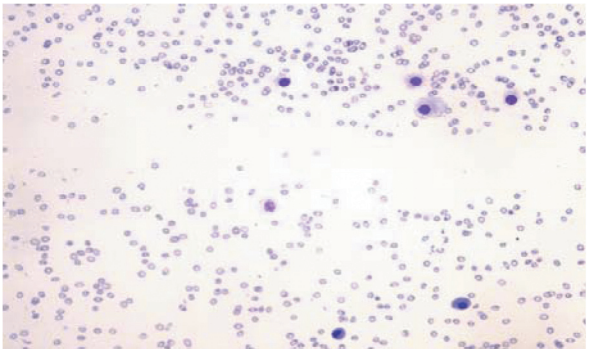


Figure 1. Mesothelial cells on a hematic background.

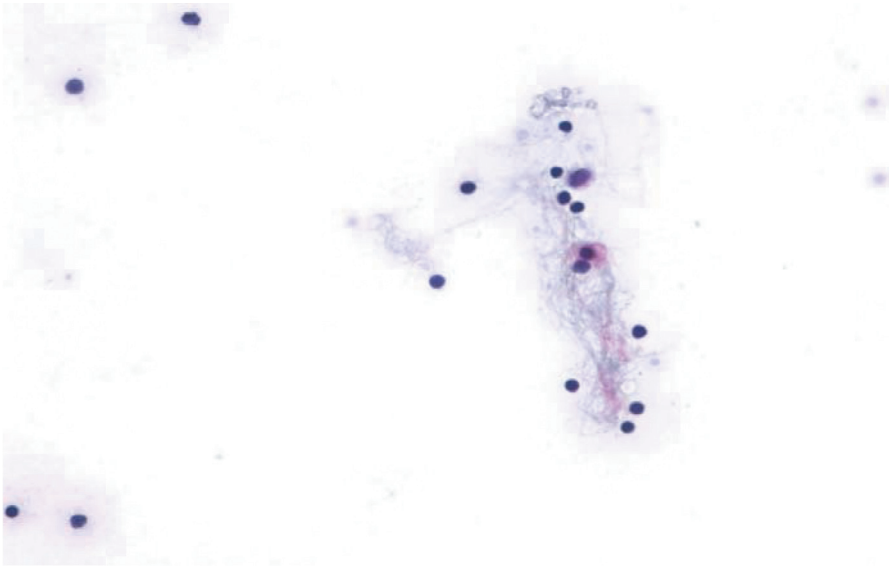


Figure 2. Reactive mesothelial cells. Papanicolaou stain X ob 40. Original

The nucleus may be eccentric, with coarse chromatin. Reactive mesothelial cells create difficulty in cytological diagnosis. It is important for cytopathological diagnosis to observe whether the atypical cells in the overgrowth are relatively uniform in size and shape, as seen in Figure 2.

## Malignant mesothelial cells in pleural fluids

In pleural effusion, differential diagnosis between proliferating mesothelial cells and malignant cells may be impossible [2]. For a good identification of malignant cells, the presence of enlarged nucleoli, abnormal mitoses, cellular elements that cannot be produced by mesothelial cells (melanin or mucus) or large cellular aggregates, papillary or glandular clusters are analysed. The absence of these landmarks, in medium-sized cells, can lead to diagnostic errors. Thus, clinical and imaging data are absolutely necessary. Atypical cells, among mesothelial cells and other cells in the overgrowth, can be easily identified by examining the smear with the microscope's wide objective. The examination reveals the quality and intensity of the overall staining of the cells and their nuclei, as well as the number of different cell populations in the smear.

The presence of malignant cells in the smear together with other elements (haematocytes, lymphocytes, polymorphonuclear cells) allows the formulation of the cytodiagnosis: positive cytology for malignancy. Fig.3.

The etiology of pleural effusions often remains unknown notwithstanding surgical pleural biopsy and further clinical observation [3]. Pleural effusions are classified as transudate or exudate effusions [4]. Certification of an effusion as transudate or exudate is given by a number of biochemical markers, including LDH enzyme, glucose, protein [5]. The LDH enzyme is an indicator of the differentiation of transudates from exudates [6].

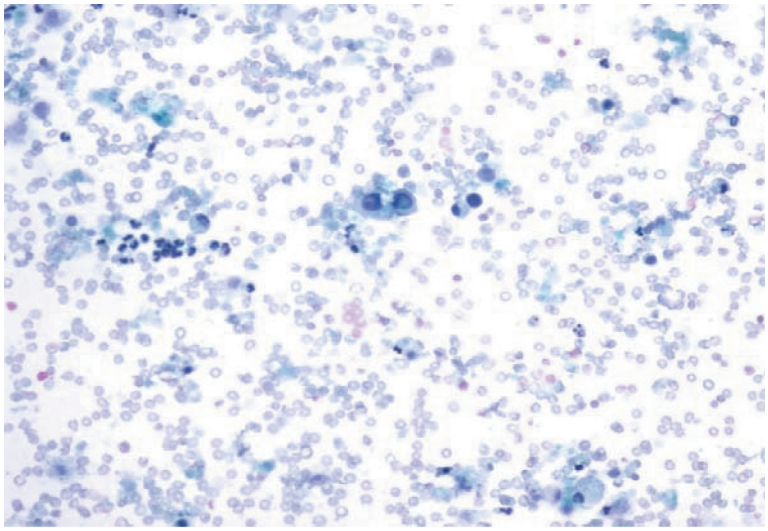


Figure 3.Papanicolaou stain Xob 40 - Malignant cells on a hematic background.

Elevated LDH levels are often associated with pleural fluid exudates and may indicate cellular damage or inflammation. Clinical practice associates elevated serum or pleural fluid LDH enzyme levels with a number of conditions, including: neoplasia, reticuloendothelial tumours, leukaemia, etc. This is because these conditions can cause cell damage or death, leading to the release of LDH into the blood and pleural fluid [6-8]. Thus, determination of LDH levels can help to assess the presence of these conditions in patients with pleural fluid above normal.

This research aims to demonstrate the relationship between pleural fluid LDH enzyme value and cytopathological outcome. The presence of lactate dehydrogenase (LDH) enzyme in pleural fluids from patients with different diseases was quantitatively assessed in conjunction with cytopathological examination.

## Materials and methods

Between 2016 and 2019, a prospective study was carried out on a group of 92 cytopathological samples analysed in the Pathological Anatomy Laboratory of the Clinical Hospital "Prof. Dr. Th. Burghele "and SC. OncoTeam Diagnostic, Bucharest, which could be correlated with the LDH enzyme values of pleural fluids from the first evacuation. The LDH enzyme values of pleural fluids from the first evacuation were collected from the Hypocrate computer system of the Clinical Hospital "Prof. Dr. Th. Burghele".

Briefly, 100 pleural fluid samples were received in the laboratory, see Koss et al [2] . The pleural fluid samples were analysed macroscopically and then transferred to disposable tubes for centrifugation at 1000 rpm for 15 minutes using the Hettich EBA 20 centrifuge.

After removal of the supernatant from the obtained sediment (1ml) conventional smears were performed.In hypocellular samples the centrifugation and supernatant removal process was repeated 3-4 times.

Two smears were performed on each sample and fixed by direct drying. From purulent or serohaemorrhagic pleural fluids a smear was made directly from the homogenised product without centrifugation, then saline was added in 1:1 solution and centrifuged. After the smears were made, 5 ml of fixative liquid (aldehyde form, 10% dilution) was added to the remaining sample to obtain the cytoblock.The smears made from the pleural fluid samples, after fixation for not more than 12 hours, were stained by the Papanicolaou or May-Grunwald Giemsa methods. To obtain the cytoblock, the sample mixture with Formaldehyde was centrifuged at 1000 rpm for 15 minutes. The supernatant was removed and the new sediment obtained was transferred to filter paper. The contents wrapped in filter paper were placed in the special work box and processed on the Donatello processor. After embedding in paraffin, 2 µm sections were made using the microtometer. The sections transferred onto slides were subjected to the deparaffinization process. One slide was stained by the hematoxylin-eosin method.

In cases with suspected malignancy, additional tests were performed by the immunocytochemical method based on the binding of an antigen, which is a cellular component, to a specific labelled antibody.

## Results

Elevated LDH levels in pleural fluid can indicate cellular damage or inflammation in the pleural space. Elevated serum or pleural fluid LDH enzyme levels can be associated with various conditions, including neoplasia, reticuloendothelial tumors, and leukemia. These conditions can lead to cell damage or death, causing LDH to be released into the bloodstream and pleural fluid. An increase in LDH levels may prompt further investigation into the underlying cause, which could include malignancies or other diseases.

The results of the 92 cytopathological samples studied were the following: 18 positive, 22 suspect and 52 negative, shown in Fig.4. (N = Negative, P = Positive, S = Suspect).

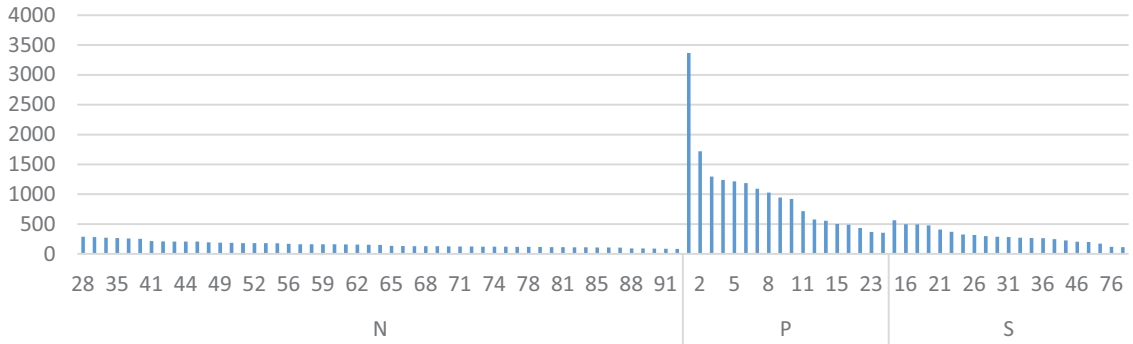
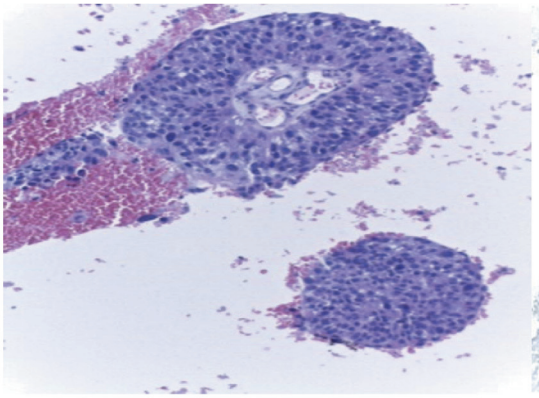
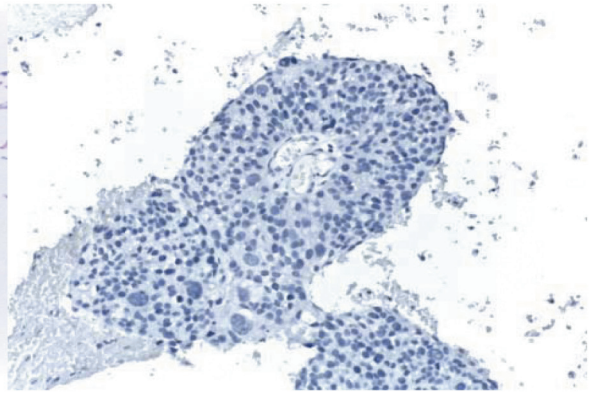



Figure 4. Correlation of LDH enzyme values with cytological result before immunocytochemical testing of suspect results.

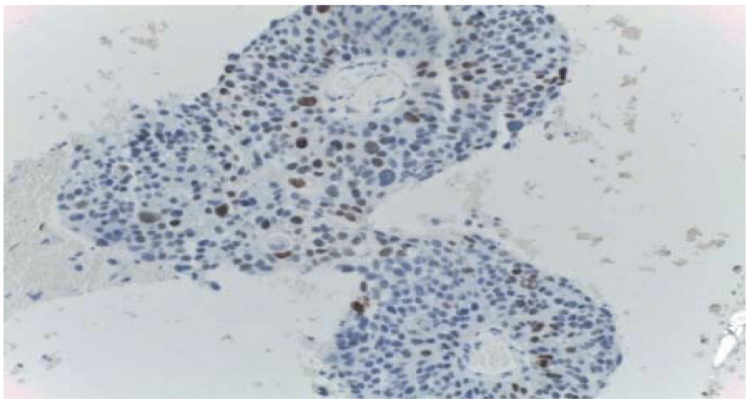
LDH levels were correlated with cytopathological aspects. Six groups of LDH values were considered; For LDH levels above 350, 24 results were identified, of which: 18 positive, 6 suspect and 0 negative. All 18 positive cytological results had LDH values above 355. At the same time, the other 6 suspicious samples were analysed with tumour markers, resulting in each diagnosis of positive cytology for malignancy (e.g. the case below);



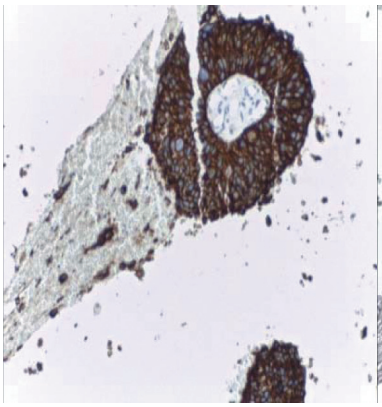
Cytoblock ob.20, Suspect



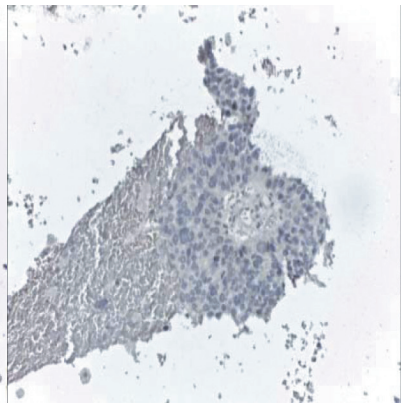
Calret ob.20, Negative



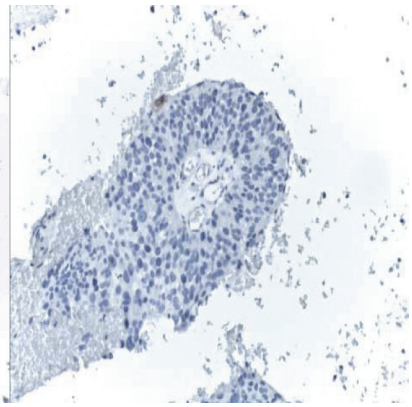
TTF1 ob.20, Positive



MCK ob.20, Positive



TRPS, ob.20, Negative

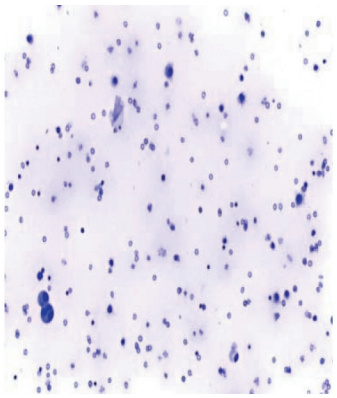


CK5-6, ob20, Negative

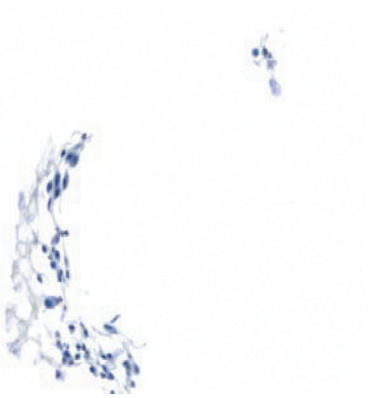
For LDH levels between 300-349, 3 results were identified, of which 0 positive, 3 suspect and 0 negative. The 3 suspect samples were analysed with tumour markers, resulting in:

- two with positive cytology diagnosis for malignancy;
- one cytologically negative for malignancy (e.g. the case on the next page);

For LDH levels between 270-299, 6 results were identified, of which 0 positive, 4 suspect and 2 negatives. The 4 suspect samples were analysed with tumour markers resulting in each diagnosis with negative cytology for malignancy; For LDH levels between 200-269, 14 results were identified, of which: 0 positive, 6 suspect and 8 negatives. The 6 suspect samples were analysed with tumour markers resulting in each diagnosis being cytology negative for malignancy; For LDH levels between 115-199, 35 results were identified, of which: 0 positive, 4 suspect and 31 negatives. The 4 suspect samples were analysed with

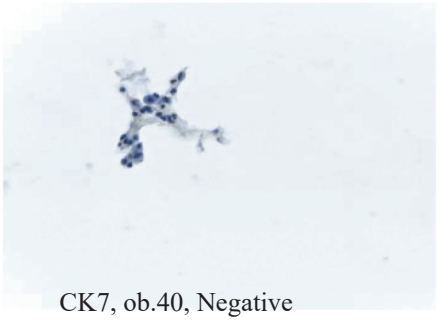


Smear, ob.20, Suspect

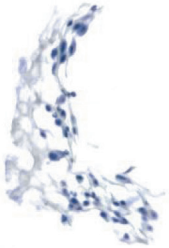


TTF1 ob.40, Negative

Calret ob.40, Negative



CK7, ob.40, Negative



WT1, ob.40, Negative

tumour markers resulting in negative cytology for malignancy at each diagnosis; For LDH levels between 50-114, 16 results were identified, of which: 0 positive, 0 suspect and 16 negatives.

## Conclusions

LDH is an enzyme with an important role in the diagnosis and evaluation of pleural fluids. Its levels can provide useful information about the nature of the pleural fluid (transudate or exudate) and may suggest the presence of certain diseases.

Comparing the positive cytopathological results with the biochemical results of the pleural fluid from the first evacuation, it was observed that there is a correlation between the positive cytopathological result and increased LDH values as shown in Figure.5.

Using the correlation of the positive cytological result, confirmed immunocytochemically for the suspected diagnostic samples, we found that these cytological results of the pleural fluid samples can be improved by the parallel determination of the LDH enzyme, thus the proportion of suspected cases becomes positive for malignancy in almost all cases with LDH values above 300. High LDH levels above 350 correlate 100% with positive cytopathological appearance for malignancy. This test can be recommended in clinical work for the detection of malignant cells in pleural effusion.

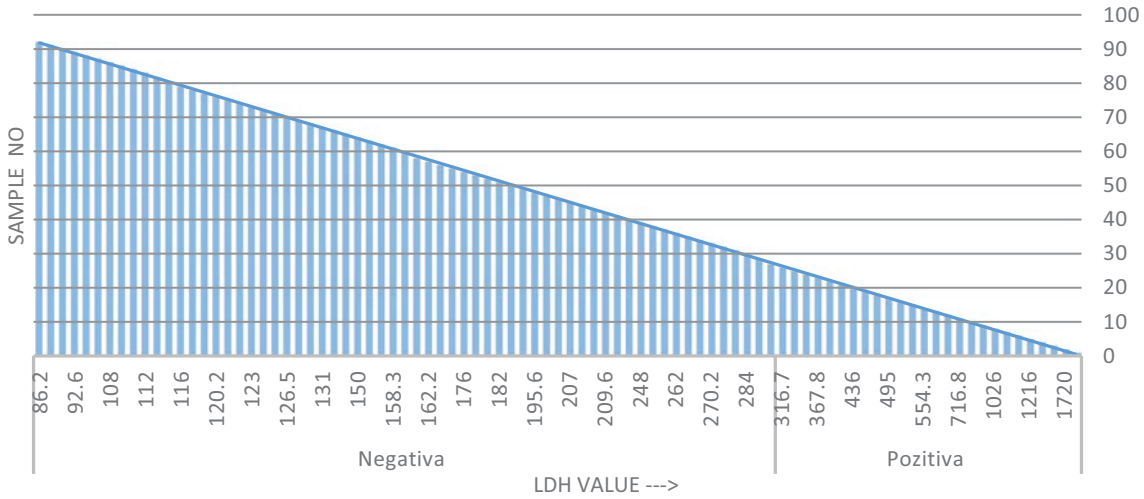



Figure 5. The correlation of LDH enzyme values with the cytological result after immunocytochemical testing of suspect results.

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