The problem of biological molecules identification is of current importance in microbiology. It has been traditionally solved by means of antibodies, containing various labels, such as enzymes, radioactive isotopes, fluorogenic or electrochemiluminescent tags. However in most cases appropriate methods are focused on identification of a significant amount of interacting molecules and don't provide insight into their spatial distribution. One of possible solutions of the problem is to use atomic force microscopy (AFM) method. AFM is a relatively new technique that has provided novel opportunities for the surface analysis of biological specimens with nanoscale resolution and minimal effect on the sample structure. The specified possibilities of AFM allow to develop highly sensitive methods for single molecule detection that opens wide prospects for the analysis of immune and substrate specific activity.

The aim of the work was the development of an AFM method for single antigen molecules identification using gold and carbonic labels. Direct visualization and quantitative evaluation of morphometric characteristics of the antigen-antibody complexes was used as a criterion for the detection.

Protein A conjugated with colloidal gold (PrA+Au) and protein G conjugated with amorphous carbon (PrG+C) were used for labeling antibodies, being bond to Rubella virus antigens onto polystyrene microtiter ELISA plates. Images were collected by using an SMM-2000 atomic force microscope (JSC "Proton-MIET Plant", Russia) operated in contact mode.

AFM-images of the antigen, antigen-antibody complexes (Ag-At); and also specific complexes Ag-At-PrA+Au and Ag-At-PrG+C were obtained step by step during the study (see Figure 1 and 2). It was shown that except specific complexes detection, the atomic force microscope allows to describe quantitatively their distribution on microtiter plate surface. Besides, the sensitivity of the method for each tag was estimated and compared to ELISA method. The obtained results indicated high sensitivity of an offered approach and certain advantages of carbonic tags use in comparison with gold tags, because of their easier detection and unambiguous identification on the received images.

Thus, offered approach in the long term perspectives will allow to solve a problem of specific marking of single molecules and microorganisms in complex multicomponent associations.

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Fig. 1: Antigen-antibody complexes labeled by colloidal gold. Scale bar is 500 nm.

Fig. 2: Antigen-antibody complexes labeled by amorphous carbon. Scale bar is 500 nm.