DOT-ELISA FOR RAPID DETECTION OF Q FEVER AND SPOTTED FEVER GROUP ANTIRICKETTSIAL ANTIBODIES

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Immune overlay assays such as ELISA are widely used for assessing the presence and/or specificity of antirickettsial antibodies for their greater speed and relative simplicity. In technical aspect there are some limitations of ELISA connected with different types of immobilizing matrices such as microtiter plates and the character of plastic as well *(1)*, considerably long time for sensibilizing the plates (*2*) and relatively large amounts of the immunoreagents required etc.

The improvement of immune overlay assays for visualizing antigens (proteins, lipopolysaccharides) bound to immobilizing solid matrices and the appearance of nitrocellulose membranes made it possible to adapt successfully the immunoenzyme test to that kind of matrix and to develop the Dot-ELISA (*3, 4*).

Dot-ELISA: two μl of the specific and control antigens diluted 1:2 were spotted on nitrocellulose paper strips (3 × 1 cm) and quenched with a solution of 5 % BSA (w/v). The specific reaction was performed after immersion of the strips in tested sera (1:100 dilution) for 90 min at 37 °C. After incubation with anti-human horseradish peroxidase (1:200) and washing, bound enzyme was detected by reaction with 4-chloro-naphtol.

Dot-ELISA revealed high level of antirickettsial antibodies in sera of patients suffering from Mediterranean spotted fever (MSF) and Q fever (phase II), and low level in the case of Q fever (phase I). Complement fixation and immunofluorescence revealed titers 1:16 - 1:32 and 1:64 - 1:512, respectively, for MSF. ELISA gave titers 1:28 - 1:512 and 1:1280 - 1:5120 for Q fever phase I and II, respectively.

The described Dot-ELISA can be used for detection of antirickettsial antibodies as it had proved to work rapidly and reliably. One of the advantages of the test is that no time is required for binding the antigens to the solid matrix as it used to be while employing microtiter plates (in some cases it lasted for 16–18 hr) (*4*). On the other hand, this test proved to be an economic one as we have needed only 2 μl amounts of purified antigen for performing a proper reaction. The requirements for sera and buffers were similar. For washing the strips it was not necessary to use a specialized technique and the reading of the results was direct according the intensity of the violet-blue colour of the spot.

The obtained results gave us the reason to believe that the Dot-ELISA can successfully find a new application in a rapid large-scale screening of human and animal sera for antibodies against Spotted Fever Group rickettsiae and *C. burnetii*.

References

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