"EVALUATION OF FLUORESCENCE POLARIZATION TEST IN DIAGNOSIS OF HUMAN BRUCELLOSIS"

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INTRODUCTION

Human brucellosis is a zoonosis that remains a worldwide veterinary, medical, and economical problem. Last 8 years, approximately 500-600 new cases of human brucellosis are registered yearly in Macedonia. Tests ranging from culture to serodiagnostic tests to the recent molecular techniques are available. Mostly used are conventional serologic techniques such as RBT (Rose Bengal, Slide Agglutination Test), Wright (Tube Agglutination Test) and Coombs (Antihuman Globulin Test), than ELISA and Competitive-ELISA. Fluorescence Polarization Assay (FPA) has been validated for number of species including cattle, swine, bison (Nielsen and Gall, ADRI, Nepean, Ont., Canada).

OBJECTIVE

Evaluation of Fluorescence Polarization Assay (FPA) in comparison with ELISA and the Conventional serologic techniques in diagnosis of human brucellosis.

MATERIAL

Patient samples were collected at 5 regional hospitals in Macedonia. Many of the patients were on treatment when blood samples were collected. Samples were held frozen at -20^{0}C until they were processed. A total of 217 sera samples were tested.

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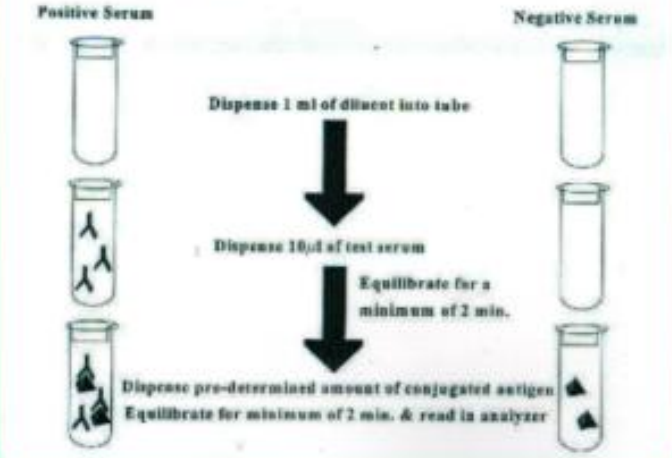
Appendix 8.1

Flowchart of Brucella FPA



FPA

METHOD

For diagnosis of brucellosis, a Fluorescence Polarization Analyzer (FPM) is used to obtain a background measurement of fluorescence of diluted serum. Antigen consisting of O-polysaccharide prepared from *B.abortus* strain 1119-3, approximately 22 kDa in size, labeled with fluorescein isothiocyanate (FITC) is added and incubated for 2 min, followed by a final reading in FPM which automatically subtracts the background reading. The net result is presented in millipolarization units (mPs).
If *brucella* specific antibodies (positive serum) are present then the result will be high mPs. In absence of anti-*brucella* antibodies (negative serum) the result is low mPs.
The cutoff for bovine sera has been tentatively set at 90 mPs.

METHOD

Initial diagnoses were made by serology: RBT, Wright and Coombs tests. To detect IgM and IgG anti-brucella antibodies NOVUM Diagnostica micro plates, coated with *Brucella* LPS antigen and reader ELISA TECAN-Classic, were used.

The basis of Fluorescence Polarization Assay (FPA) is that a molecule in solution rotates randomly at a rate inversely proportional to its size. If the molecule is labeled with a fluorescent marker and is examined by plane polarized light, a small molecule will rotate through a given angle faster than a larger molecule. The time of rotation may be measured using horizontal and vertical measurements.

RESULTS

	Sensitivity %	Specificity %
Wright	82	98
Coombs	89	100
ELISA	98	100
FPA*	98	92

* Cut-off that correlates with this results was 80 mPs

CONCLUSIONS

✓ **FPA, as an accurate, relatively inexpensive, simple and very rapid test, is a very promising tool in diagnosis of human brucellosis beside diagnosis in animals.**

✓ **Further studies concerning best cut-off for human samples, sensitivity and specificity are needed.**

✓ **ELISA remains a reference method in serologic diagnosis of human brucellosis.**