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# **Diagnosis of Visceral Leishmaniasis: A Review**

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**ABSTRACT:** The diagnosis of visceral leishmaniasis involves isolation of the parasites in culture or invasive and risky techniques including demonstration of parasite in stained preparations of relevant tissues such as spleen, bone marrow or lymph node by light microscopic examination remains a gold standard. Serological tests using rK39 in ELISA or rapid immunochromatographic tests, direct agglutination test and immunoblotting which are based on the detection of antileishmanial antibodies are costly, require sophisticated techniques.

**KEY WORDS:** Visceral leishmaniasis, direct agglutination test, Indirect Fluorescent Antibody Test

## **I.INTRODUCTION**

Clinical features of VL can be easily mistaken for other febrile illnesses such as malaria and enteric fever. Reliable laboratory methods become mandatory for accurate diagnosis. Early case detection followed by adequate treatment is central to the control of VL [1,2]. Patient management, screening of asymptomatic infections, surveillance including verification of elimination, and epidemiological studies are some of the areas where diagnostic tests play a major role. Ideally, a test should make the distinction between acute disease and asymptomatic infection, as most of the antileishmanial drugs are toxic. Moreover, such tests should be highly sensitive and specific, simple and affordable.

Visceral leishmaniasis can be confirmed by microscopical examination of stained slides of spleen, bone marrow or lymph node aspirates. The amastigote forms (called LD bodies) can be seen in tissue smears from lymph nodes, bone marrow or spleen. Splenic smears have the sensitivity of 93.1–98.7% [3]. Bone marrow and the lymph node smears have lower sensitivity ranging from 52–85% and 52–58% [3] respectively. Bone marrow aspiration (BMA) or splenic aspirations are painful and risky techniques. Serious or fatal bleeding after splenic aspiration is not uncommon, however, in skilled and experienced hands, serious bleeding is rare. The use of microscopy in the diagnosis of VL offers the benefits of high specificity. But like all microscopic procedures it suffers from variability of detection, sensitivity and the inevitable need for an expert microscopist. Culture, too, suffers from the same deficiencies and the tedious, time consuming nature of the technique and the high cost are prohibitive and thus, except in dedicated research laboratories, it is seldom used for clinical diagnosis. In a modification in the form of microtitre culture, sensitive and reproducible detection of parasites was possible using buffy coat (WBC rich layer) and peripheral blood mononuclear cells (PBMC) isolated from patient blood [4].

Several tests have been developed to detect antibodies against *Leishmania* in blood or serum of VL patients. However, some tests are not appropriate for field use such as Indirect Fluorescent Antibody Test (IFAT) and Enzyme Linked Immunosorbent Assay (ELISA). IFAT is based on detecting antibodies, which are demonstrated in the very early stages of infection and are undetectable six to nine months after cure. If the antibodies persist in low titres, it is an indication of a probable relapse. It is sensitive (96%) and specific (98%) but the requirement of sophisticated laboratory conditions prohibit its application in the field [5]. ELISA has also been used in the serodiagnosis of VL but its sensitivity and specificity depends on the antigen used.

Moreover, immunochromatographic strips using K39 antigen are also available to aid in the diagnosis of VL [6]. In direct agglutination test (DAT), Coomassie brilliant blue stained whole promastigotes are incubated with sera of the patients and agglutination observed after an overnight incubation. It is a highly specific, sensitive, inexpensive and simple test. Initially, aqueous antigen was used but it had the drawback of cold chain requirement, and short life. Now, freeze dried antigen has been developed which can be transported at ambient temperature. The major disadvantage of DAT is the need of multiple pipetting, relatively long incubation time and high cost of antigen. As with any antibody



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based test, DAT remains positive for a long time after the disease is cured, and thus cannot be used as a test of cure or for diagnosis of relapses [7].

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