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The diagnostic utility of real-time quaking-induced conversion assay (RT-QuIC) in the diagnosis of sporadic Creutzfeldt-Jakob disease

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Sporadic Creutzfeldt-Jakob disease (sCJD) belongs to a family of fatal neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs) or prion diseases. Neuropathologically these diseases are characterized by the post-translational conformational change of a normal cellular protein called prion protein (PrP^{C}) into a disease-associated form, termed PrP^{Sc}. PrP^{Sc} is partially protease-resistant and can induce PrP^{C} to undergo a conformational change and produce more PrP^{Sc} in a self-propagating manner by a seeded aggregation process. Current diagnostic criteria for sCJD rely on clinical features and the results of EEG, MRI and the presence of 14-3-3 in the cerebrospinal fluid (CSF). These tests are not specific for sCJD. A new approach to the pre-mortem diagnosis of sCJD has been to exploit the ability of small amounts of CSF PrP^{Sc} to convert native PrP into PrP^{Sc} in a newly described protein aggregation assay known as real-time quaking induced conversion (RT-QuIC). In 2012 we reported that a retrospective study showed that CSF RT-QuIC had a sensitivity of 89% and a specificity of 99% for sCJD. Results of a prospective audit of CSF RT-QuIC in the United Kingdom showing that it has a sensitivity of 90% and a specificity of 100% will be presented and the role of RT-QuIC in the clinical diagnosis of sCJD described.

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Detection of infection with *Actinomyces israelii* by monoclonal antibodies 101-121 against *Actinomyces israelii* polysaccharide

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Human actinomycosis, is rare a chronic, suppurative granulomatous infectious disease, has been recognized for over a century, caused by microorganisms of genus *Actinomyces* species. Presently, *Actinomyces* species comprises 42 species, 20 of them are relevant to human medicine. Actinomycosis is defined as a hard mass-type lesion with a specific histopathological structure. There are a large number of case reports of actinomycosis in the literature, but in most cases, diagnosis has been based solely on clinical and histopathological findings. The goal of the study was to determine the chemical composition of polysaccharide antigens extracted from *A. israelii* and generate monoclonal antibody reactive with the polysaccharide to understand their role in pathogenicity. Polysaccharides were extracted from dry bacterial cell mass by using trichloroacetic acid (TCA) and enzymes (DNase, RNase and protease). Further were purified by ion-exchange chromatography (DEAE Sephadex A25) and gel filtration (TOYOPEARL HW 55 s). Composition and structure of polysaccharide was determined by gas liquid chromatography-mass spectrometry GLC-MS. Monoclonal antibodies were generated by the hybridoma technique. The ELISA method was carried out for evaluating specificity of monoclonal antibody to the polysaccharide antigen. Then quantitative immunoprecipitation test has been performed. The experimental infection of mice with *A. israelii* was performed and investigated in histological study. Detection of the bacterial strain within the mouse tissues was done by immunohistochemical test. Scanning electron microscopy showed a variation of the branched shape of *A. israelii* with filamentous character of slime. Polysaccharide of *A. israelii* consists of glucose, galactose and mannosamine in the molar ratio 1, 5, 10 respectively. Two hybridomas 101 and 121 producing mAbs against polysaccharide antigen were IgM class. Quantitative microimmunoprecipitation test showed that monoclonal antibody precipitated polysaccharide antigen of *A. israelii*. Immunohistochemical test with monoclonal antibodies identified *A. israelii* infection in the liver mouse tissue. Monoclonal antibodies with polysaccharide used in immunohistochemical assays could serve as tools for diagnostic purposes *in vitro* approaches.

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