New method for counting and sizing clinically relevant microparticles (MPs) in the 0.15 to 1 micron size range.

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MPs are fragments of cell membranes that are generated following activation or apoptosis. Clinical interest in MPs is based on a potential diagnostic role in thrombotic and vascular disorders. Development of analytic approaches has been limited by the lack of instrumentation to detect particles below 0.5µ, the conventional limit of flow cytometry. Invitrox Sizing, Antigen Detection and Enumeration (ISADE) is a Mie scattering method that does not require any assumption for particle shape or calculation of a diffusion coefficient. Particle size is determined directly from the intensity of light scattered at a defined scattering angle. Single particles are assessed one at a time. ISADE can assess MPs from 0.15 to 20µ. MPs from different cellular sources are easily analyzed in plasma samples. In contrast to the sharp size distributions seen with NIST standard beads, biological MPs are highly polydisperse. Several different broad-based populations of MPs are often observed in clinical samples. Populations in the 0.2 to 0.3µ and 0.35 to 0.6µ range are typical. Sample preparation is critical in size analysis of MPs; centrifugal fields (CF) <1,000g provide best results while minimizing MP aggregation. MPs appear to be stable to freeze-thaw provided mechanical shear is avoided. Recent use of ISADE to size lipid vesicles showed polydispersity in vesicle size. Clinical diagnostic applications are promising. Analysis of 40 acute liver failure samples showed particles in the 0.7 to 1.25µ range in all patients, likely representing incomplete removal of platelets or large platelet fragments. All samples contained a second MP population in the 0.3 to 0.6µ range, the number of which varied from patient to patient, but was relatively constant from day to day in the same patient. A third MP population was seen in most samples in 0.1 to 0.2µ range. Of interest was a MP population in the 0.24 to 0.29µ range that was present in some patients. Although preliminary, it appeared that these individuals had more severe disease. We conclude that ISADE is a convenient and reliable instrument to analyze MP numbers and size distribution in clinical samples.