In an effort to build internal combustion engines (ICE) with both reduced brake-specific fuel consumption (BSFC) and better emission control, engineers developed the direct injection spark ignition (DISI) engine. DISI engines combine the specific higher output power of the spark ignition engine with the better efficiency of the compression ignition engine at part load. Despite their benefits, DISI engines still suffer from high hydrocarbon, NOx and particulate matter (PM) emissions. Until recently, PM emissions have received relatively little attention, despite their severe effects on human health, which are related mostly to their size. Previous research indicates that almost 80% of the PM is emitted during the first few minutes of the engine’s operation (cold-start-fast-idling period). A proposed solution for PM emission reduction is the increase of the ethanol content in the fuel. This research measures experimentally the effect of ethanol content in the fuel on the PM formation in the combustion chamber from a DISI engine during the cold-start period. A novel sampling system has been designed and combined with a SMPS in order to measure the particulate matter number (PN) distribution from only 15 cm after the exhaust valves of a modern DISI engine, for a temperature range from 0 to 40°C, under low load operation. Seven gasohol fuels have been tested with the ethanol content varying from 0% (or E0) to 85% (or E85). For E10 to E85, PN increases modestly when the engine coolant temperature (ECT) is lowered. The PN distributions, however, are insensitive to the ethanol content of the fuel. The total PN for E0 is substantially higher than the gasohol fuels, at ECT below 20°C. However, for ECT higher than 20°C, the total PN values (obtained from integrating the PN distribution from 15 to 350 nm) are approximately the same for all fuels. This sharp change in PN from E0 to E10 is confirmed by running the tests with E2.5 and E5. The midpoint of the transition occurs at approximately E5. Because the fuels’ evaporative properties do not change substantially from E0 to E10, the significant change in PN is attributed to the particulate matter formation chemistry.