Title: Impact of particle size of ground corn samples on the detection of the presence of 35S and TNOS sequences.

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Accurate analysis of the adventitious presence of a genetically modified organism (GMO) is crucial for meeting certain market and export guidelines. One influence on the accuracy can be the particle grind size of the sample. A research study of different particle grind sizes analyzed across four or more laboratories will provide insight on DNA detection capabilities. Event-free corn base material (EFBM) was verified by subsampling three pools of 1000 seed and screening for the detection of 35S and TNOS with real-time PCR using in-house validated methods. A spike material (SM) for corn event MON-88017 was verified on 90 individual seeds using ELISA methods for Cry3Bb protein detection. The EFBM and SM was then ground and sieved to separate the ground material into two particle sizes (<1mm, 1mm-2mm). Using the sieved material, a 0.4% and 0.9% spike level sample for each particle size were prepared on a % w/w basis for a 400 and 200 seed pool, respectively. For detection of the 0.4% spike level, each laboratory received 10 tubes of each particle size containing 1.0 gram of ground material. Eight of the ten tubes contained a subsample of the 0.4% spike level while the remaining tubes contained a subsample of the EFBM. For the detection of the 0.9% level, each laboratory received 6 tubes of each particle size containing 1.0 gram of ground material. Three of the six tubes contained a subsample of the 0.9% spike level while the remaining tubes contained a subsample of the EFBM. Participating laboratories extracted the samples for DNA analysis using their in-house validated real-time PCR methods for the detection of 35S and TNOS. Qualitative results were reported and summarized by SoDak Labs. Based on the dataset, neither particle size impacted detection for TNOS at the 0.9% level for any of the laboratories, nor for 35S at the 0.9% level for two of the labs. For both particle sizes, a false positive was detected for 35S at the 0.9% level for one of the laboratories, however it was commented Cq values were close to negative threshold. At the 0.4% level, a false positive was detected for the <1mm particle size by one laboratory. Both laboratories correctly identified negative subsamples at the 1mm-2mm spike level. The limit of detections was reported at 0.03% and 0.01%. This research was funded in part by a grant from the Seed Testing Research Foundation. Thank you to AgReliant Genetics, LLC for their participation.