Objective: The aim of this in vitro study was to evaluate the effects of clear and opaque fissure sealants on readings of laser fluorescence (LF) and light-emitting diode (LED) based caries detection devices. Background data: When planning patient care, the practitioner needs to consider any changes in the status of the sealed surface for the long-term success of the sealant. As visual inspection is difficult to perform on sealed surfaces, adjunct diagnostic methods must be used to improve follow-up assessments and increase the accuracy of caries diagnosis. Methods: Forty-six freshly extracted permanent human molars were selected and divided into two groups. Each group was treated with a different sealant (clear and opaque). The teeth were measured twice by two blinded observers using an LF-based and an LED-based device before and after sealing. The data were analyzed using Wilcoxon’s matched-pairs signed-rank test and a paired t-test. Cohen’s $j$ and the intraclass correlation coefficient were used to examine intra- and inter-examiner repeatability. Results: The values of the LED device were significantly higher after the application of the opaque sealant, but there was no statistically significant difference after the application of the clear sealant ($p = 0.15$). The LF-based device readings were also significantly lower after both the clear and the opaque sealant applications ($p < 0.001$). Conclusions: The readings from the LF-based device were affected by both sealants. The readings from the LED-based device were affected by the opaque sealant but not by the clear sealant.