Background
The SENTiFIT® system (SENTiFIT assay) composed of the SENTiFIT® 270 Analyser, the FOB Gold® reagents and the SENTiFIT® pierceTube, is a specific system for the detection of human hemoglobin present in feces, known as the FOB (focal occult blood) test. The SENTiFIT assay is a quantitative FIT test (Fecal immunochemical Test), highly recommended for both routine diagnostic testing and the CRC (ColoRectal Cancer) screening program.

Purpose of the study
The aim of our study was to evaluate the Clinical Performances of SENTiFIT® FOB Gold® Latex test, using SENTiFIT® 270 System (SENTiFIT assay), comparing it with the OC SENSOR DIANA System (DIANA assay) already in use in the lab, both FIT quantitative assays.

Methods:
Patients:
Overall, 134 patients were enrolled in our prospective pilot clinical study. Fecal samples were taken from adults for routine outpatient diagnostic workup, and subsequent monitoring for the detection of human hemoglobin in our Clinical Microbiology Laboratory. All patients were informed by a clinical provider about the aims of the study and were provided with two sets of 3 sample collection tubes each: one set containing 3 OC-Auto Sampling Bottle and one set containing 3 SENTiFIT® pierceTube (Figure 2).

Sample collection:
The sample collection tubes were numbered (1 to 3) in each set. Patients were asked to collect 3 different bowel motions, following the sequence of the sample collection tube numbers and using both sets. Moreover, a clinical provider emphasized the importance of collecting samples from different areas of the feces with both sticks and then wiping the tube sticks against each other in a circular movement for fecal homogenization (Figure 3).

Cutoff level:
As initial approach for results analysis, we used the DIANA cutoff level (100ng/mL) to define positive results for both assay. Subsequently, we evaluated other two cutoff values for the SENTiFIT assay, as followed:

• 117 ng/mL, which corresponds to 100 ng/mL of DIANA assay, considering the difference in buffer volume in SENTiFIT® pierceTube (Table 2);

• 107 ng/mL, which corresponds to the best sensitivity and specificity after ROC curve analysis (Table 2 and Figure 4).

Statistical elaboration of experimental results - MedCalc Statistical Software version 16.4.2 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2016)

Results:
A total of 387 collection tubes of the DIANA assay and 387 collection tubes of the SENTiFIT assay were considered adequate for the study (15 collection tubes from each assay were eliminated from the study due to inadequate collection procedure). Based on the DIANA assay cutoff level of 100 ng/mL, 28 samples were detected as positive and 324 samples were detected as negative by both assays - concordance of 93.5% (Table 1 and 2). 23 samples (6.48%) gave discrepant results, 24 samples were detected as positive by the SENTiFIT assay and negative by the DIANA assay, whereas, only one sample was detected as positive by the DIANA assay and negative by the SENTiFIT assay. Significant correlation between SENTiFIT assay and DIANA assay, with a sensitivity of 96.6% and specificity of 93.3% was obtained using the same cut off of DIANA system (100ng/mL) for both assays (Table 1, Table 2). Moreover, the maximum values of sensitivity (96.6%) and specificity (93.3%) were obtained using 107 ng/mL cutoff level for the SENTiFIT assay (Table 2 and Figure 4).

Discussion and Conclusions
Herein, we report the results of a unique clinical comparison evaluation study of SENTiFIT assay using the SENTiFIT® 270 automated system. For the first time, we conducted a clinical study on a large group of patients, undergoing routine diagnostic FIT testing, using 2 different sets of collection tubes for 3 bowel movements each. Three different cutoff levels gave high concordance and AUC results, as shown in table 2.

Although significant importance was given to sample collection and fecal homogenization instructions, we could hypothesize that discrepant results, as already shown by others, can be explained by sample collection variability (performed by the patient at home), feces heterogeneity, hemoglobin stability and the use of antibodies anti-human hemoglobin against different epitopes. Unfortunately, we could not further investigate and confirm discrepant samples by endoscopic examination as patients were followed up elsewhere.

In conclusion, findings in current study show good concordance between the SENTiFIT assay and the DIANA assay and therefore, we consider the SENTiFIT assay a valid quantitative Fecal Immunochemical Test. We believe the results above should stimulate further studies using larger number of patients.

Disclosure of interest:
RD received financial support from Sentinel Diagnostics for the submitted study; We declare No financial relationships with any other organization that might have an interest in the submitted study in the previous three years; We declare No other relationships or activities that could appear to have influenced the submitted study.