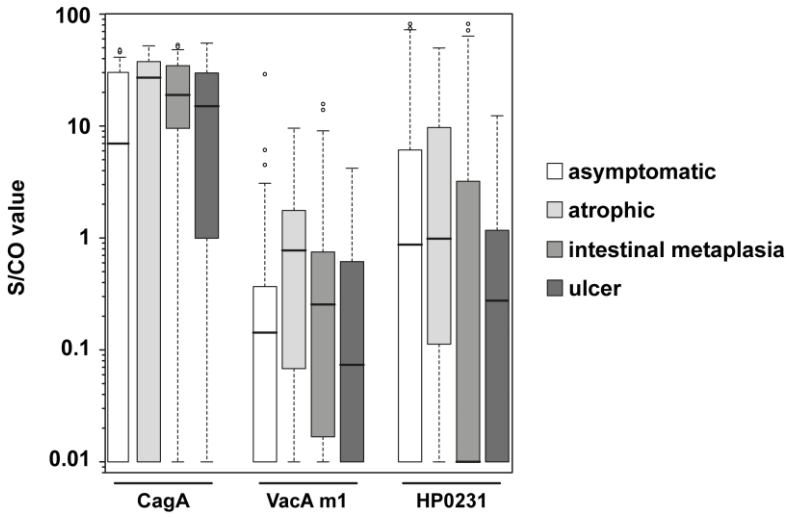

Figure 3. Boxplots of S/CO values of CagA, VacA m1 and HP0231 in different subgroups of HP positive samples. Every box represents the interquartile range and the horizontal line inside the box is the median. Whiskers show the 5th and the 95th percentiles. Outliers are plotted as circles. S/CO values of IgG responses towards CagA, VacA m1, and HP0231 are shown for *H. pylori* positive asymptomatic samples (n = 66; white boxes), *H. pylori* positive atrophic samples (n = 18; light gray boxes), *H. pylori* positive intestinal metaplasia samples (n = 41; gray boxes) and *H. pylori* positive ulcer samples (n = 14; dark gray boxes).

4. Discussion

During assay development, the incubation times for the samples and the detection antibody were optimized in order to keep them as short as possible, without a loss of sensitivity. The microfluidic system allows a short assay protocol, with a 35 min assay incubation time in total. This is less than one third of the assay time needed in other commercially available immunoblot systems. Besides a high technical assay precision, very good results were achieved for specificity and sensitivity of the selected antigens. The *H. pylori* lysate showed the best sensitivity (99.3%) and specificity (100%) in this study, and the overall performance was better compared to other commercial tests (claimed in the test manual), which also use a *H. pylori* lysate, e.g., anti-Helicobacter pylori IgG (Orgentec Diagnostika GmbH, Main, Germany) or Helico Blot 2.1 (MP Biomedicals Asia Pacific Pte Ltd., the Cavendish Singapore Science Park, Singapore). In comparison to another multiplex serological assay by Michel et al. [22], which showed 89% sensitivity and 82% specificity, we received higher sensitivity and specificity for FliD and the *H. pylori* lysate. The other antigens for sample classification used in our assay yielded lower sensitivity (82.0–87.1%) than Michel et al. [22], but higher specificity (95.2–97.4%). The clinical classification as positive or negative for an *H. pylori* infection of these samples was determined by Warthin–Starry silver stain, after taking a biopsy, and not by serology. Therefore, a comparison of sensitivity and specificity of our assay with a non-invasive clinical classification method (e.g., ELISA) was not possible. However, sensitivity and specificity of the different *H. pylori* antigens and the lysate in our assay should be confirmed with another set of samples positive and negative for *H. pylori*.

FliD showed the best sensitivity (95.7%) as a single antigen, but also, 2 of 63 *H. pylori* negative samples were reactive to FliD, giving the specificity of 96.8%. Exactly these two samples showed also reactivity towards one other *H. pylori* antigen. Therefore, we suspect that these two *H. pylori* negative patients had a former *H. pylori* infection, which was eradicated or lost a long time ago. The *H. pylori* lysate is probably not sensitive enough to detect this low reactivity. However, a reduced sensitivity for CagA and VacA m1 was expected due to the fact that these antigens are virulence factors, and not expressed by each *H. pylori* strain [23].