Know thy Helicobacter pylori with ddPCR

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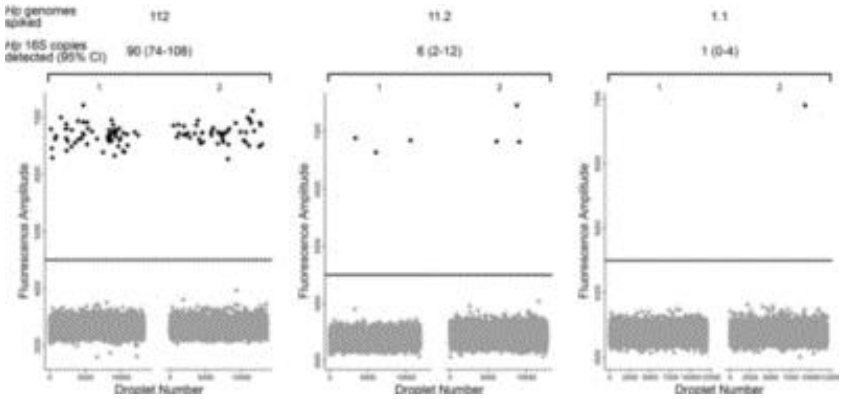
A Neves



Droplet digital PCR amplitude plot from stool DNA devoid of H. pylori spiked with three concentration of H. pylori genomic DNA. Each dot represents one droplet and dots above the line are considered to be positive for the H.pylori 16s gene.

Image provided by Dr. Sarah Talarico

Approximately half of the world's population is infected with the gastric bacterium *Helicobacter pylori (H. pylori)*. While some cases of *H. pylori* infection can lead to gastric cancer, most infections remain either asymptomatic or can even be protective in esophageal cancer or asthma. A combination of environmental factors and genetics of both the host and pathogen contribute to disease outcome, so determining the strain characteristics of *H. pylori* present in infected individuals is an important goal of molecular epidemiology studies. However, current strategies for detecting and genotyping *H. pylori* require an endoscopy of the upper gastrointestinal tract, which prevents sampling from asymptomatic individuals. Therefore, there is an urgent need to develop effective non-invasive methods for genotyping *H. pylori*. A new Fred Hutch study led by Dr. Sarah Talarico, an associate in Dr. Nina Salama's Laboratory (Public Health Sciences, Basic Sciences and Human Biology Divisions) and published in *Helicobacter*, accomplished just that by developing and validating a non-invasive, stool-based method for genotyping *H. pylori*.

A key feature of the study was a recent development in Polymerase Chain Reaction (PCR) technology, named droplet digital PCR (ddPCR), that uses a water-oil emulsion system to partition a 20 microliter reaction into thousands of nanoliter-sized droplets. With this protocol, at most one DNA fragment is present in any droplet. The PCR reaction is carried out within each droplet and by determining the frequency of positive droplets, allows a quantitative readout. To develop the assay,

the researchers obtained matched stool and serum from 50 volunteers from a Costa Rican cohort as well as stool samples from 29 individuals that had been admitted to Harborview Medical Center in Seattle for various medical reasons. To test the performance of the assay, the authors introduced known quantities of *H. pylori* DNA into a stool DNA sample form an *H. pylori* negative volunteer. These experiments revealed a clear separation between droplets positive for the 16S ribosomal gene (a gene that is specific for each bacterial species) and droplets that lacked the 16S gene (see figure). The presence of the *cagA* gene in *H. pylori* and its allelic variants is associated with increased risk of developing gastric cancer. For example, the CagA protein is phosphorylated on an amino acid motif, EPIYA, which can classify a strain as either having an EPIYA-C or an EPIYA-D motif, with the latter being linked to increased gastric cancer risk. The *cagA* specific ddPCR correctly identified and determined the EPIYA type in 15 out of 16 strains tested (94%). Next, the researchers compared their ddPCR assay with other non-invasive assays such as the *H. pylori* serum antibody test. The ddPCR assay detected *H. pylori* in the stool of 27 out of 32 Costa Rican participants who were positive for serum antibodies and in 12 out of 12 that were positive in the stool antigen test from the Harborview participants. Intriguingly, there was considerable variation in the *H. pylori* load in both the Costa Rican and US samples, wherein samples that tested positive for CagA tended to have higher loads. Said Dr. Talarico "The quantitative nature of the droplet digital PCR method revealed variation in *H. pylori* load in the stool. Those infected with an *H. pylori* strain having the *cagA* virulence gene had a significantly higher *H. pylori* load, a correlation that has also been observed in the stomach. We are conducting additional studies to better understand the relationship between *H. pylori* load in the stomach and in the stool. We are excited to continue adapting this new stool-based method for future molecular epidemiologic studies to look at other *H. pylori* genotypes, including antibiotic resistance alleles, other virulence genes, and to determine strain ancestry. Since this non-invasive assay can be used even for the majority of *H. pylori*-infected individuals who are asymptomatic, this opens up many more study design possibilities". In summary, this study developed an assay to detect and genotype *H. pylori* that is both quantitative and non-invasive, and warrants further validation by using it with matched gastric biopsy samples from diverse populations.

Talarico S, Safaeian M, Gonzalez P, Hildesheim A, Herrero R, Porras C, Cortes B, Larson A, Fang FC, Salama NR. 2015. Quantitative detection and genotyping of *Helicobacter pylori* from stool using droplet digital PCR reveals variation in bacterial loads that correlates with cagA virulence gene carriage. *Helicobacter*. Doi: 10.1111/hel.12289. [Epub ahead of print.]

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