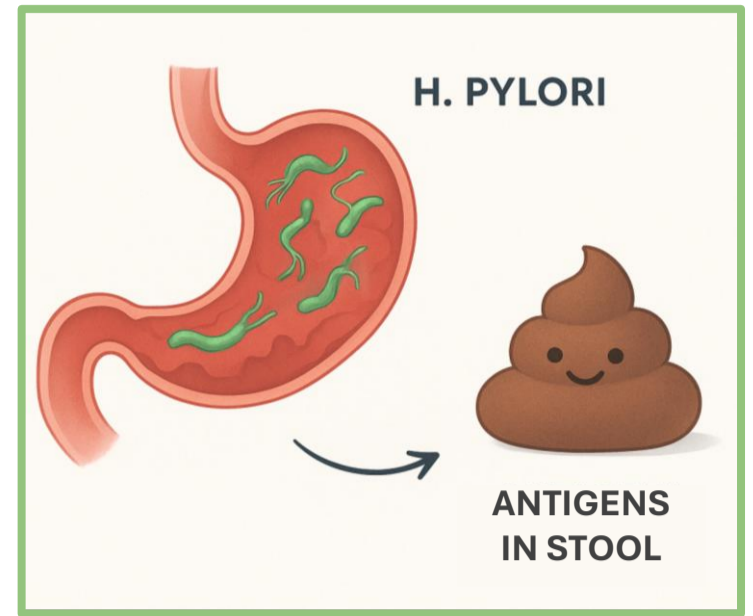


Validation of a New Automated Method for the Detection of *H. pylori* Fecal Antigen

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Introduction

The detection of *Helicobacter pylori* antigen in stool samples represents a non-invasive diagnostic method of high clinical relevance for identifying infection caused by this bacterium. *H. pylori* is a pathogen associated with several gastrointestinal diseases, including gastritis, peptic ulcers, and gastric cancer. Traditionally, procedures such as gastroscopy with biopsy and culture, or tissue urease testing, are considered the gold standard, although they involve invasive and costly approaches. In this context, non-invasive tests based on the detection of *H. pylori* antigen in feces have become first-line diagnostic tools. Fecal antigen testing enables the identification of active infection, monitoring of therapeutic response, and confirmation of eradication after treatment.



VS.



Aim of the study

At the Biallisi laboratory, results obtained using the current in-house method for *H. pylori* antigen detection (card test, Beta Diagnostici) were compared with those from a new turbidimetric assay- soon to be available - automated on the SENTiFIT® 270 Analyzer (PYLiaGold™ turbidimetric assay, Sentinel Diagnostics, Milan).

Materials and Methods

A total of 678 routine samples were analyzed, 20% of which had previously tested positive with the card method. Samples were resuspended using a dedicated collection device (PYLiaGold™ pierceTube). Each sample was tested simultaneously with the card method and the SENTiFIT® 270 Analyzer, and each tube was tested in duplicate using two different reagent lots, yielding a total of four determinations per sample.



Results

After excluding test runs in which IQCs were out of range, 1965 total measurements were considered. These data demonstrated that the two reagent lots displayed comparable performance in terms of sensitivity (Table 2). Because an improvement in performance was observed throughout the study—linked both to operator handling and the “freshness” of the analyzed samples—the analytical performance was assessed initially on a dataset of 372 results and subsequently on a smaller subset of 93 samples. All results were analyzed using different cut-off values between 0.47 and 0.51 ng/mL, and the corresponding Cohen’s kappa coefficient was calculated. The analysis yielded a Cohen’s kappa of 0.63, indicating substantial agreement between the two methods. With a cut-off of 0.51 ng/mL, the positive percent agreement (PPA) was 92.5% and the negative percent agreement (NPA) was 89.8%. Using a cut-off of 0.47 ng/mL, the PPA increased to 95% while the NPA remained at 89.8% (Table 1).

k Cohen =0,61		Beta D. Cards			
Cutoff 0,51 ng/mL		Pos	Neg		
PYLiaGold™	Pos	37	34	71	
	Neg	3	298	301	
		40	332	372	
OPA	90,1%				
NPA	89,8%				
PPA	92,5%				

k Cohen =0,63		Beta D. Cards			
Cut off 0,47 ng/mL		Pos	Neg		
PYLiaGold™	Pos	38	34	72	
	Neg	2	298	300	
		40	332	372	
OPA	90,3%				
NPA	89,8%				
PPA	95,0%				

Tab. 1: Total results 93 samples x 2 fecal samplings x 2 lots = 4 results

k Cohen =0,61		Beta D. Cards			
Cut off 0,47 ng/mL		Pos	Neg		
PYLiaGold™ Lot 1 Tube 1	Pos	9	8	17	
	Neg	1	75	76	
		10	83	93	
OPA	90,3%				
NPA	90,4%				
PPA	90,0%				

k Cohen =0,61		Beta D. Cards			
Cut off 0,47 ng/mL		Pos	Neg		
PYLiaGold™ Lot 2 Tube 1	Pos	10	10	20	
	Neg	0	73	73	
		10	83	93	
OPA	89,2%				
NPA	88,0%				
PPA	100,0%				

k Cohen =0,64		Beta D. Cards			
Cut off 0,47 ng/mL		Pos	Neg		
PYLiaGold™ Lot 1 Tube 2	Pos	10	9	19	
	Neg	0	74	74	
		10	83	93	
OPA	90,3%				
NPA	89,2%				
PPA	100,0%				

k Cohen =0,65		Beta D. Cards			
Cut off 0,47 ng/mL		Pos	Neg		
PYLiaGold™ Lot 2 Tube 2	Pos	9	7	16	
	Neg	1	76	77	
		10	83	93	
OPA	91,4%				
NPA	91,6%				
PPA	90,0%				

Tab. 2: Tube 1 vs. Tube 2 : Two samplings tested with two reagent lots = 1 result per sample/lot/tube.

Conclusions

The automated PYLiaGold™ turbidimetric assay by Sentinel Diagnostics enables automated and therefore more standardized sample processing, demonstrating an overall analytical reproducibility greater than 90% (NPA 89.8% and PPA 95%). The optimal cut-off providing the best balance between sensitivity and specificity was identified as 0.47 ng/mL.

However, the study highlighted the critical importance of pre-analytical sample handling, particularly ensuring that samples are collected and processed within the timeframes specified by the manufacturer, and that testing is performed by adequately trained personnel.