SIMPLE AND RAPID DETECTION OF HELICOBACTER PYLORI ANTIGEN IN FECAL SAMPLES

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ABSTRACT

Introduction. Helicobacter pylori (Hp) is classified as class I carcinogen by WHO and may impact atrophic gastritis, ulcerous diseases and is related to gastric cancer and MALT-lymphoma. The prevalence of Hp infection in the Czech Republic is about 42%. ^{13}C-urea breath test is accepted as gold diagnostic standard, alternatively Hp antigen detection in stool can be used.

Aims & Methods. This study evaluates two Hp-antigen rapid tests: helicoCARE direct (Care Diagnostica, Austria) and RAPID Hp StAR™ (Oxoid - DAKO), both was compared with ELISA test Amplified IDEIA™ Hp StAR™ with monoclonal antibodies (Oxoid - DAKO). The comparison was performed on 50 stool specimens stored at -70°C. The preanalytical process of both tests were compared as well.

Results. The matching results of helicoCARE direct with ELISA test occurred in 42 cases (Cohen's κ = 0.672, 95% CI = 0.465 - 0.878), relative sensitivity, specificity and accuracy to ELISA test were 79%, 94% and 84%. RAPID Hp StAR™ test matched with ELISA test in 30 specimens (Cohen's κ = 0.543, 95% CI = 0.312 - 0.775), relative sensitivity, specificity and accuracy to ELISA test were 64%, 100% and 76%.

Conclusions. The preanalytical phase is more confident in RAPID Hp StAR™ test, where extracts are prepared by examiner by about 100 mg of stool. In helicoCARE test are extracts prepared by patient, more easily, compared to RAPID Hp StAR™, but wholly independently to the laboratory. The analytical accuracy and agreement with ELISA test were higher for helicoCARE direct test.

RAPID Hp StAR™

Test *RAPID Hp StAR™* - rapid amplified immunochromatographic assay for qualitative determinatin of Hp antigen in stool. Rapid test uses the same monoclonal antibodies as ELISA ***Amplified IDEIA™ Hp StAR™***. Diagnostic sensitivity - 97.6 %, and specificity 94.2 %.

The Rapid HpStAR test doesn't include any patient sampling system.

Patient sampling - to plastic container with spoon extractor fixed to container cap. Stool samples were stored - refrigerated at -75°C.

Laboratory procedure - 100 mg of stool samples was weighed, extracted to buffer solution and left for 12 hours at 4 - 8°C to next day. Using the disposable plastic pipette provided 350µL of the stool suspension was added to the test tube.

Hp TEST COMPARISON

κ = 0,543

		ELISA IDEIA™ Hp StAR™ neg.	posit.	Σ
RAPID Hp StAR™	neg.	17	12	29
	posit.	0	21	21
	Σ	17	33	50

κ = 0,672

		ELISA IDEIA™ Hp StAR™ neg.	posit.	Σ
helicoCare direct	neg.	16	7	23
	posit.	1	26	27
	Σ	17	33	50

concordance of both tests κ = 0,605

		helicoCare direct neg.	posit.	Σ
RAPID Hp StAR™	neg.	21	8	29
	posit.	2	19	21
	Σ	23	27	50

Relative sensitivity, specificity and accuracy (compared to ELISA method)

RAPID Hp StAR™ - 64 %, 100 %, 76 %

helicoCARE direct - 79 %, 94 %, 84 %

Amplified IDEIA™ Hp StAR™

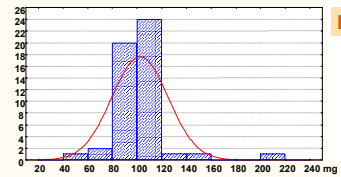
Amplified IDEIA™ Hp StAR™ - qualitative test with diagnostic sensitivity 95.3 -100 % and specificity 96.9–99.4 %; sandwich ELISA test utilises highly specific monoclonal antibodies combined with a polymer dextran conjugate.

Patient sampling - to plastic container with spoon extractor fixed to container cap. Stool samples were stored - refrigerated at -75°C.

Laboratory procedure - 100 mg of stool samples were weighed, extracted to buffer solution pH 7.4, centrifugated 30 minutes and left for 12 hours at 4 - 8°C to next day. ELISA method was run according to manufacture instructions.

Evaluation of ELISA microplates was performed using vertical ELISA 8-channel reader Spectra, at wavelengths 450 and 650 nm.

STOOL SAMPLING

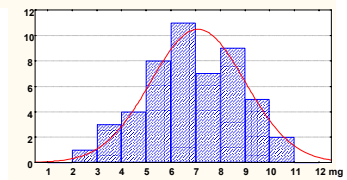


RAPID Hp StAR™

- histogram of 50 samples
- 100 mg weight in laboratory
- 50 mg as a half amount positivity of test remain
- 200 mg as double amount hook-effect not found

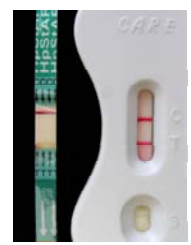
helicoCare direct

- histogram of 50 samples
- 10 mg weight in laboratory
- < 5 mg as a half amount false negative in 2/5 tests
- > 200 mg as double amount hook-effect not found



Hp TEST COMPARISON

helicoCare negative
Hp StAR negative



helicoCare positive
Hp StAR negative

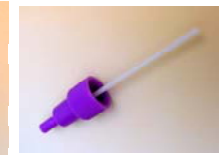
helicoCare positive
Hp StAR positive

helicoCARE direct

Test *helicoCARE direct* - immunochromatographic assay for qualitative determinatin of Hp antigen in stool with analytical sensitivity 4–8 ng Hp antigen/ml. Diagnostic sensitivity - 89 %, specificity 96 %.

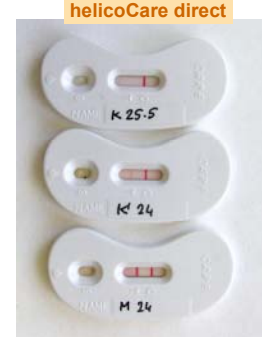
Patient sampling - to plastic container with brush extractor fixed to container cap. Only the amount of stool, that remains in the tread of the applicator, should be put into the collection tube with extraction buffer.

Laboratory procedure - breaking the tip of cassette and adding two drops of sample solution to the cassette.



TEST EVALUATION

helicoCare direct



RAPID Hp StAR™

CONCLUSIONS

- **Stability of Hp antigen** in all stool samples stored and refrigerated at –70 °C remains stable during tested 5 years.
- **Quantity of sampled stool** affects rapid tests results at minimal range. Doubling of stool sampling will not cause antibody saturation, and didn't display false negative results. Stool extraction with very high sample concentration didn't display false positivity. Using just half amount of stool sample may cause false negative results.
- **Rapid tests** are much quicker compared to ELISA method, it is possible to examine one sample only, namely right by the doctor, test does not require laboratory equipment.
- **Results of both rapid tests agreed** in 80 %, concordance rate expressed coefficient κ was 0,605. Higher concordance with ELISA method we found with helicoCARE direct, κ = 0,672, RAPID Hp StAR™ test embodied with ELISA method ELISA coincident only in 76 % results, concordance rate κ = 0,543.
- **RAPID Hp StAR™** tests could be aesthetically evaluated like affable, in light of ergonomy was we found better **helicoCARE direct** tests. helicoCARE direct tests could be influenced much more by patient stool sampling and has higher pre-analytical errors. RAPID Hp StAR™ tests are fully prepared by laboratory and pre - analytical phase is therefore more reliable.
- **Expenses** are comparable in both rapid tests.

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