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#### Please use only the valid version of the package insert provided with the kit.

#### **INTENDED USE**

Enzyme Immunoassay (ELISA) for the determination of Helicobacter pylori Antigen (H Pylori Ag) in human stools.

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

#### PRINCIPLE OF THE TEST

Microplates are coated with a cocktail of affinity purified mouse monoclonal antibodies directed to the most specific Helicobacter pylori antigens.

In the 1<sup>st</sup> incubation, the solid phase is treated with the sample, previously extracted from stools, and simultaneously with a mixture of monoclonal antibodies to Hp, conjugated with peroxidase (HRP).

After washing out all the other components of the sample, in the  $2^{nd}$  incubation the bound enzyme specifically present on the solid phase generates an optical signal that is proportional to the amount of H. pylori antigens present in the sample.

#### **COMPONENTS**

H. Pylori Ag contains reagents to perform 48 tests.

#### 1. Microplate [MICROPLATE]

 $n^{\circ} 1 - 6$  strips x 8 breakable microwels, coated with anti HP Ag specific affinity purified mouse monoclonal antibodies and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

#### 2. Standards [CAL..]

n° 4 vials - Lyophilized standards.

To be dissolved with EIA grade water (please refer to the vial label).

When dissolved, Standards have the following concentrations:  $0 - 0.1 - 0.5 - 1.0 \,\mu g/mL HP Ag$ 

They contain fetal bovine serum, inactivated HP Ag, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives.

Important Note: Standards when dissolved are not stable. Proceed as described in the proper section for storage.





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#### 3. Wash buffer concentrate 20X [WASHBUF 20X]

1 x 60 mL/bottle - 20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.05% Kathon GC.

#### 4. Enzyme Conjugate [CONJ]

1 x 8 mL/vial - Ready to use component. It contains Horseradish Peroxidase (HRP) labeled mouse monoclonal antibodies to HP Ag , 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives. The Enzyme Conjugate is color coded red.

#### 5. Chromogen/Substrate Solution [SUBS TMB]

1 x 16 mL/vial - It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide ( $H_2O_2$ ). *Note: To be stored protected from light as sensitive to strong illumination.* 

#### 6. Specimen Diluent [DIL SPE]

1 x 60 mL/vial - Buffered solution for the extraction of HP Ag from the specimen and preparation of the sample. It contains 10 mM Tris-HCl buffer pH 7.4+/-0.1, 2% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives. The component is color coded blue.

#### 7. Sulphuric Acid [H2SO4 0.3M]

1 x 10 mL/vial - It contains 0.3 M  $H_2SO_4$  solution. Attention: Irritant (Xi R36/38; S2/26/30)

**8. Plate sealing foils:** n° 2

9. Package insert: n° 1

#### **Upon request:**

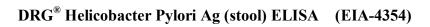
### H. Pylori Ag Extraction kit REF EIA-4355

This kit contains all what is necessary to prepare n° 48 samples extracted from stools collected by samples.

#### MATERIALS REQUIRED BUT NOT PROVIDED

- 1. Calibrated variable volume Micropipettes ranging 1000 µL and 200 µL; disposable plastic tips.
- 2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
- 3. Timer with 60 minute range or higher.
- 4. Absorbent paper tissues.



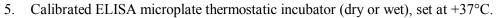


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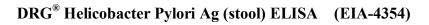


- 6. Calibrated ELISA microwell reader with 450 nm (reading) and possibly with 620-630 nm (blanking) filters.
- 7. Calibrated ELISA microplate washer.
- 8. Vortex or similar mixing tools.
- 9. Disposable plastic micro-spoon stools collection container (available on request from DRG)

#### WARNINGS AND PRECAUTIONS

- 1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
- 2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
- 3. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
- 4. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
- 5. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
- 6. Check that the liquid components of the kit are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
- 7. Avoid cross-contamination between samples by using disposable tips and changing them after each sample.
- 8. Avoid cross-contamination between kit components by using disposable tips and changing them between the use of each one.
- 9. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
- 10. Treat all specimens as potentially infective, according to national regulations and laws concerning biological sample handling and wasting.
- 11. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
- 12. Wastes produced during the use of the kit have to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min.
- 13. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.





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- 14. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
- 15. Other waste materials generated from the use of the kit (example: tips used for samples and controls, tools for the extraction of the sample from specimens, used microplates, etc.) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

*Important Note*: Degradation of HP antigen heavily occurs in stools after 24 hrs generating false negative results, even if the specimen is stored at 2-8°C.

The next following operations are described and represented in figures in the Instructions for Use of the Stool Extraction Kit provided together with the kit.

Operate according to the following instructions:

- 1. Open the stool collection device and introduce the extraction brush deeply into the specimen. Rotate the brush 2-4 times in order to collect the right amount of biological material (about 0.2 gr).
- 2. Transfer the brush carefully into the test tube supplied in the kit and then add 1 mL Specimen Diluent. Keeping the brush inside the tube, mix vigorously on vortex for 2 min +/-10% in order to dissolve H. pylori into solution.
- 3. Discard the brush and insert the filtering piston, supplied with the kit, into the tube. Push gently the piston down into the tube in order to collect not more than 150-200  $\mu$ L of the liquid phase of the suspension, volume enough to carry out the test.

#### Important Notes:

- *a.* Be careful not to apply a too strong manual pressure on the piston. The piston could break the tube and spills could be generated. If this should happen, use a paper towel soaked with an hospital disinfectant to clean up the contaminated surfaces.
- **b.** Avoid any addition of preservatives to samples, especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.

#### PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

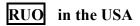
#### **Microplates:**

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storing. In this case, call DRG's customer service.

Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2-8°C.

*Important Note*: *After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.* 

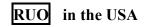








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#### Standards:

Add the volume of ELISA grade water reported in the label to the lyophilized powder of each Standard. Let fully dissolve the content and then gently mix on vortex.

*Important Note*: When dissolved, Standards are not stable. Store Standards frozen in aliquots at  $-20^{\circ}$ C, carefully labeled with the content of HP Ag present in each of them.

#### Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with ELISA grade water and mixed gently end-overend before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

*Important Note:* Once diluted, the wash solution is stable for 1 week at  $+2-8^{\circ}$  C.

#### Enzyme Conjugate:

Ready to use. Mix well on vortex before use.

#### **Chromogen/Substrate Solution:**

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, and if possible, sterile disposable container.

#### **Specimen Diluent:**

Ready to use. Mix well on vortex before use.

#### Stop Solution (Sulphuric Acid):

Ready to use. Mix well on vortex before use. Attention: Irritant (Xi R36/38; S2/26/30) Legenda: R 36/38 = Irritating to eyes and skin. S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

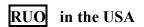
#### INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

- 1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of  $\pm 2\%$ .
- 2. The ELISA incubator has to be set at  $+37^{\circ}$ C (tolerance of  $\pm 0.5^{\circ}$ C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.





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- 3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/standard and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 300µl/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls/standard and well-characterized negative and positive reference samples, and check to "Internal Quality Control". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions provided by the manufacturer. *Important Note:* Due to the nature of the sample used and the possible presence of particles in the sample, be careful to control that the needles of the washer do not get blocked by the presence of stool bodies.
- 4. Incubation times have a tolerance of  $\pm 5\%$ .
- 5. The ELISA microplate reader has to be equipped with a reading filter of 450 nm and ideally with a second filter (620-630 nm) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.
- 6. When using an ELISA automated workstation, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.

*Important Note:* Due to the nature of the sample used and the possible presence of particles in the sample, be careful to control that the needles of the workstation do not get blocked by the presence of stool bodies. We strongly suggest to use disposable sample tips in order to avoid any block or damage of fix probes.

7. Upon request, DRG offers a sample preparation device able to produce a particle free sample showing excellent performances in the assay. Please inquire.

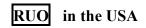
#### PRE ASSAY CONTROLS AND OPERATIONS

- 1. Prepare the sample from stools as described in section G and represented in the Instructions for Use of the H. Pylori Ag Extraction Kit.
- 2. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.





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- 3. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
- 4. Dilute all the content of the 20x concentrated Wash Solution as described above.
- 5. Dissolve the Standard Set as described above.
- 6. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
- 7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
- 8. Check that the ELISA reader has been turned on at least 20 minutes before reading.
- 9. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
- 10. Check that the micropipettes are set to the required volume.
- 11. Check that all the other equipment is available and ready to use.
- 12. In case of problems, do not proceed further with the test and advise the supervisor.

#### ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Two procedures are available: a quantitative method able to provide a quantification of HP Ag in the specimen and a qualitative method.

Quantitative Assay

- 1. Place the required number of strips in the plastic holder and carefully identify the wells for standards and samples. Leave A1+B1 wells empty for blanking purposes.
- 2. Pipette 100 µL Standards in duplicate into the calibration wells (see the example of dispensation reported below).
- With the Pasteur pipette supplied aspirate the liquid filtered up into the inner chamber of the piston and dispense 3 drops (about 100 μL) of sample into the sample well.
   Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm. (samples show OD values higher than 0.100).
- 4. Dispense then 100 µL Enzymatic Conjugate in all wells, except for A1+B1, used for blanking operations.

*Important note:* Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.





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5. Following addition of the conjugate, check that the color of the samples have turned from brown to pale reddish and incubate the microplate for **120 min at +37°C**.

*Important notes:* Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA workstations.

- 6. When the first incubation is over, wash the microwells as previously described (section 9.3)
- Pipette 200 μL Chromogen/Substrate into all the wells, A1+B1 included. Incubate the microplate protected from light at room temperature (18-24°C) for 20 min.

Important note: Do not expose to strong direct light as a high background might be generated.

- Pipette 100 μL Stop Solution into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 7.
- 9. Measure the color intensity of the solution in each well, as described in section 9.5 using a 450 nm filter (reading) and if possible a 620-630 nm filter (background subtraction), blanking the instrument on A1 or B1 or both.

An example of dispensation scheme is reported below:

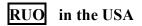
Microplate						
	1	2	3	4	5	6
Α	BLK	Std. 4				
В	BLK	Std. 4				
С	Std. 1	Sample 1				
D	Std. 1	Sample 2				
Е	Std. 2	Sample 3				
F	Std. 2	Sample 4				
G	Std. 3	Sample 5				
Н	Std. 3	Sample 6				

Legenda: BLK = Blank, Std = Standard





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Qualitative Assay

- 1. Place the required number of strips in the plastic holder and carefully identify the wells for standards and samples. Leave A1well empty for blanking purposes.
- 2. Pipette 100 μL Standard 1 in duplicate, 100 μL Standard 2 in duplicate, 100 μL Standard 4 in single and then 100 μL samples. Check for the presence of samples in wells as reported before.
- 3. Dispense 100 µL Enzymatic Conjugate in all wells, except for A1, used for blanking operations.

*Important note:* Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.

4. Following addition of the conjugate, check that the color of the samples have turned from brown to pale reddish and then incubate the microplate for  $120 \text{ min at } +37^{\circ}\text{C}$ .

*Important notes:* Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA workstations.

- 5. When the first incubation is over, wash the microwells as previously described (section 9.3)
- 6. Pipette 200 μL Chromogen/Substrate into all the wells, A1 included. Incubate the microplate protected from light at room temperature (18-24°C) for 20 min.

Important note: Do not expose to strong direct light as a high background might be generated.

- 7. Pipette 100 μL Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 6.
- 8. Measure the color intensity of the solution in each well, as described in section 9.5 using a 450 nm filter (reading) and if possible a 620-630 nm filter (background subtraction), blanking the instrument on A1.

An example of dispensation scheme is reported below:





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Microplate						
	1	2	3	4	5	6
Α	BLK	Sample 3				
В	Std. 1	Sample 4				
С	Std. 1	Sample 5				
D	Std. 2	Sample 6				
Е	Std. 2	Sample 7				
F	Std. 4	Sample 8				
G	Sample 1	Sample 9				
Н	Sample 2	Sample 10				

Legenda: BLK = Blank, Std = Standard

#### Important notes:

- 1. If the second filter is not available, ensure that no fingerprints or dust are present on the external bottom of the microwell before reading at 450nm. They could generate false positive results on reading
- 2. Reading should ideally be performed immediately after the addition of the acid solution but definitely no longer than 20 minutes afterwards. Some self-oxidation of the chromogen can occur leading to a higher background.





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#### ASSAY SCHEME

Operations	Procedure
Standards & samples	100 µL
Enzyme Conjugate	100 µL
1 <sup>st</sup> incubation	120 min
Temperature	+37°C
Washing steps	n° 4-5
Chromogen/Substrate	200µl
2 <sup>nd</sup> incubation	20 min
Temperature	room
Sulphuric Acid	100 µL
Reading OD	450 nm

#### INTERNAL QUALITY CONTROL

A check is performed on the controls/standard any time the kit is used in order to verify whether the expected OD 450nm or S/Co values have been matched in the analysis.

Ensure that:

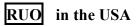
Parameter	Requirements
Blank well	< 0.100 OD 450nm value
Std. 0 µg/mL	< 0.200 mean OD 450nm value after blanking
Std. 0.1 µg/mL	OD 450nm > OD 450nm Std. 0 µg/mL + 0.100
Std. 1 µg/mL	> 1.000 OD 450nm value

If the results of the test match the requirements stated above, proceed to the next section. If they do not, do not proceed any further and perform the following checks:





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Problem	Check
Blank well	1. that the Chromogen/Substrate solution has not become contaminated during the
> 0.100 OD 450nm	assay
<b>Std. 0 μg/mL</b> > 0.200 OD 450nm	1. that the washing procedure and the washer settings are as validated in the pre qualification study;
after blanking	2. that the proper washing solution has been used and the washer has been primed with it before use;
	3. that no mistake has been done in the assay procedure (dispensation of positive standards instead of the negative one);
	<ol> <li>that no contamination of the standard or of the wells where the standard was dispensed has occurred due to spills of positive samples or of the enzyme conjugate;</li> </ol>
	5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate
	6. that the washer needles are not blocked or partially obstructed.
Std. 0.1 μg/mL	1. that the procedure has been correctly performed;
OD 450nm < Std. 0 μg/mL +	2. that no mistake has occurred during its distribution (ex.: dispensation of negative standard instead)
0.100	3. that the washing procedure and the washer settings are as validated in the pre qualification study;
	4. that no external contamination of the standard has occurred.
Std. 1 µg/mL	1. that the procedure has been correctly performed;
< 1.000 OD 450nm	2. that no mistake has occurred during the distribution of the standard (dispensation of negative standard instead).
	3. that the washing procedure and the washer settings are as validated in the pre qualification study;
	4. that no external contamination of the standard has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.





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#### **CALCULATION OF RESULTS**

Quantitative Assay

Calculate the mean OD 450nm value of the standards. Then draw a calibration curve possibly using a 4 parameters fitting curve system. Then calculate on the curve the concentration of HP antigen in the sample.

Qualitative Assay

The test results are calculated by means of a cut-off value determined from the O 450nm value of the Std. 0  $\mu$ g/mL (Std. 0) and the OD 450nm of the Std. 0.1  $\mu$ g/mL (Std. 0.1) with the following formula:

#### Cut-Off = (Std. 0 + Std. 0.1) / 2

**Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.

#### BIBLIOGRAPHY

- 1. Fujisawa T. et al. J Clin Lab Anal. 2001; 15(3):154-9 Braden B. et al. Ann Med. 2001 Mar; 33(2):91-7
- Dominguez-Bella MG. Et al. FEMS Microbiol Lett. 2001 Apr 20;198(1):15-6 Ding HJ. Et al. J Gastroenterol. 2001 Apr; 36(4):237-41
- 3. Monteiro L. et al. J Microbiol Methods. 2001 Jun; 45(2):89-94
- 4. Kim N. et al. Korean J Intern Med. 2000 Dec; 15(3):187-94
- 5. Monteiro L. et al. Am J Gastroenterol. 2001 Feb; 96(2):353-8
- 6. Shimada T. et al. Nippon Rinsho. 2001 Feb; 59(2):280-5
- 7. Brown LM. Epidemiol Rev. 2000; 22(2):283-97
- 8. Ameriso SF. Et al. Stroke 2001 Feb; 32(2):385-91
- 9. Vaira D. et al. Aliment Pharmacol Ther. 2000 Oct; 14 suppl (3):13-22