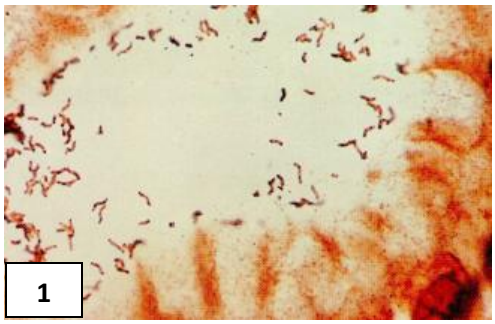
Unit V – Problem 4 – Microbiology: Helicobacter pylori

- **Helicobacter pylori:**
  - It is a gram-negative bacterium able to survive and colonize the human stomach.
  - This bacterium causes infection in 30-40% of the population in developed countries and 50-80% of population in developing countries.
  - Eradication of H.pylori significantly reduces duodenal and gastric ulcer relapse.
- **Pathogenecity factors of H.pylori:**
  - **Bacterial adhesion.**
  - **Vacuolating cytotoxin (vacA).** This toxin inserts into the membrane of gastric epithelial cells resulting in the formation of channels which will result in water influx, vesicle swelling and vacuole formation.
  - **Cytotoxin-associated gene (cagA)** probably associated with more severe forms of the disease.

    Notes:
    - ✓ *Not all stains of H.pylori produce these pathogenecity factors and this explain why the presence of the organisms per se does not imply gastric disease.*
    - ✓ *H.pylori is linked to gastric adenocarcinoma (6 fold risk increase) and MALT lymphoma (Mucosal Associated Lymphoma Tissue).*
    - ✓ *Antibiotic resistance is increasing and is a major cause of concern.*
    - ✓ *Infection with H.pylori results in an acute inflammatory reaction:*
      - ❖ *IL-8 recruiting neutrophils which will release oxidative radical and proteolytic enzymes. Notice that evasion of the host inflammatory reaction by H.pylori leads to chronic active gastritis.*
- **Pathogenecity factors of H.pylori (more details):**
  - The bacterium is acquired via the oral route.
  - H.pylori survives the low pH of the stomach because of the protective action of urease (which the bacterium produces). Urease buffers the pH around the bacterial surface.
  - The bacterium, propelled by powerful flagella and helped by the helicoidal shape, penetrates into the mucus layer which is covering the superficial stomach cells.
  - H.pylori adheres strongly to the apical cell surface via adhesions and (vacA) and induces a rearrangement of the underlying cytosol.
- **Diagnosis of H.pylori:**

Invasive tests (endoscopy-based)	Non-invasive tests
Rapid urease test	13-14-C-urea breath test
Direct microscopy	Serology (IgG, IgA)
Histology	PCR in saliva and feces
Culture	
DNA probes/ PCR	

- **Images (1) and (2) show H.pylori with silver stain:**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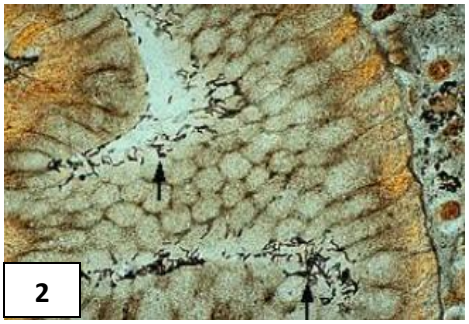
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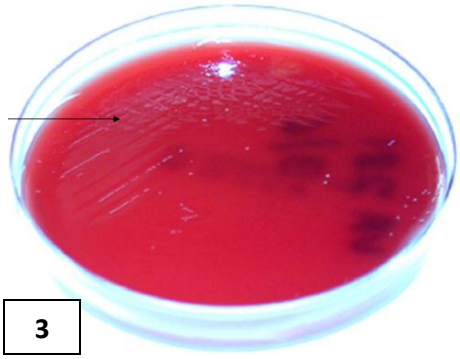
- **Cultivation of H.pylori:**
  - **Image (3):** Petri dish with H.pylori growth at 48 hours on Trypticase Soy Agar (TSA) + 5% sheep blood. The bacteria was isolated from a gastric biopsy.



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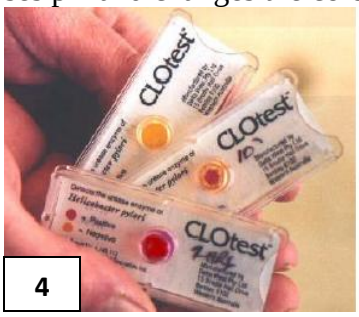
- **Rapid biopsy urease test (CLOtest: image-4):**
  - **What do different colors mean?**
    - ✓ Yellow = negative test or un-used test.
    - ✓ Red = infection with H.pylori.
  - **How does biopsy urease test differ (image-5)?**
    - ✓ Urease enzyme of H.pylori present in the biopsy causes release of ammonia. This raises pH and changes the color of pH indicator from yellow to magenta.



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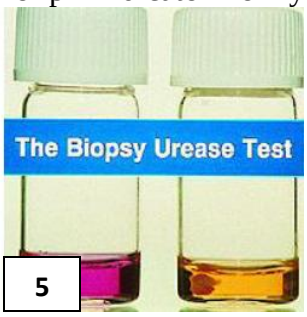

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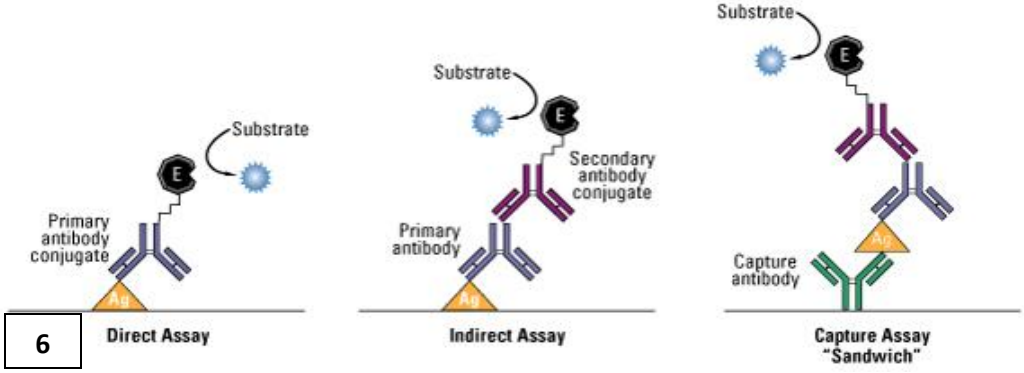
- **Enzyme-Linked Immunosorbant Assay (ELISA: image-6):**
  1. Plate wells are sensitized with antigen.
  2. Antibody is added to bind to the antigen.
  3. Ligand (linking molecule) with enzyme added to bind to the antibody.
  4. Substrate is added to give color after reacting with enzyme.
  5. Stop reagent is added.
  6. End product = antibody amount is measured by optical density scanning.



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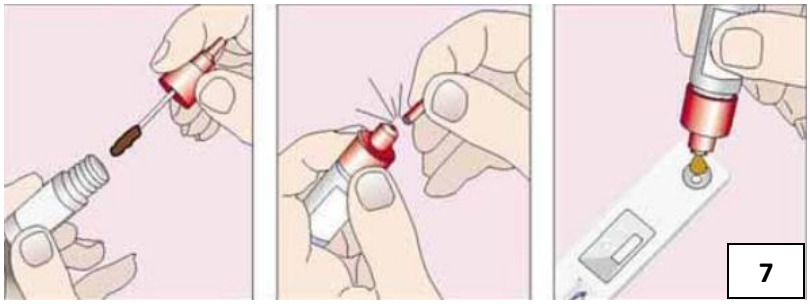
- **Immunocard for detection of H.pylori agents in stool (image-7):**
  1. Using the applicator stick of the diluents vial, transfer a small portion (5-6 mm diameter) of stool specimen into the sample diluent.
  2. Vortex for 15 seconds.
  3. Dispense 4 drops into the round window at the lower end of the device and read the result after 5 minutes.
  - **Interpretation of results:**
    - ✓ Negative: one blue line (control).
    - ✓ Positive: one blue line (control) and one pink-red line (test).



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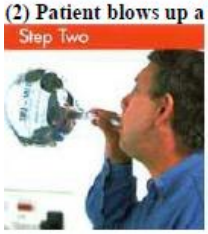
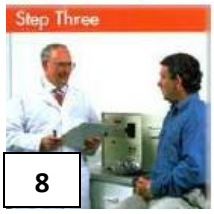
- **^{13}C urea breath test (image-8):**

(1) Patient takes the capsule

(2) Patient blows up a balloon



(3) Breath sample analyzed



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