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Diagnosis and treatment of *Helicobacter pylori* infections.

Real-time PCR for *H. pylori* diagnosis. Better than culture?

Leif Percival Andersen, MD

Copenhagen University Hospital

Denmark.

Culture of *H. pylori*.

- **In the literature culture or histology detects 30-90% of the expected *H. pylori* positive gastric biopsies.**
- **A higher detection rate of *H. pylori* is obtained in focused projects than under routine conditions.**
- **Several culture media and staining methods are available.**

Genomic detection of *H. pylori*.

- **Conventional PCR**
  - **One primer**
- **Nested PCR**
  - **2 - 4 primers (different virulence gens)**
- **Real-time PCR**
  - **Quantitative PCR**
- **Luminex PCR**
  - **Several primers**
- **Sequencing**
  - **16s / 23s / whole genome**

Conventional PCR.

- A *Helicobacter* genus specific or a *Helicobacter pylori* specific amino acid sequence is selected as primers.
- Well known virulence gens as *hsp60*, *ure*A, *urea*C or *Hp*16s are often used for *H. pylori* PCR detection.
- Different primers may have different sensitivities and specificities.

Concordance between PCR, culture and histology.

Six studies have compared conventional PCR and culture.

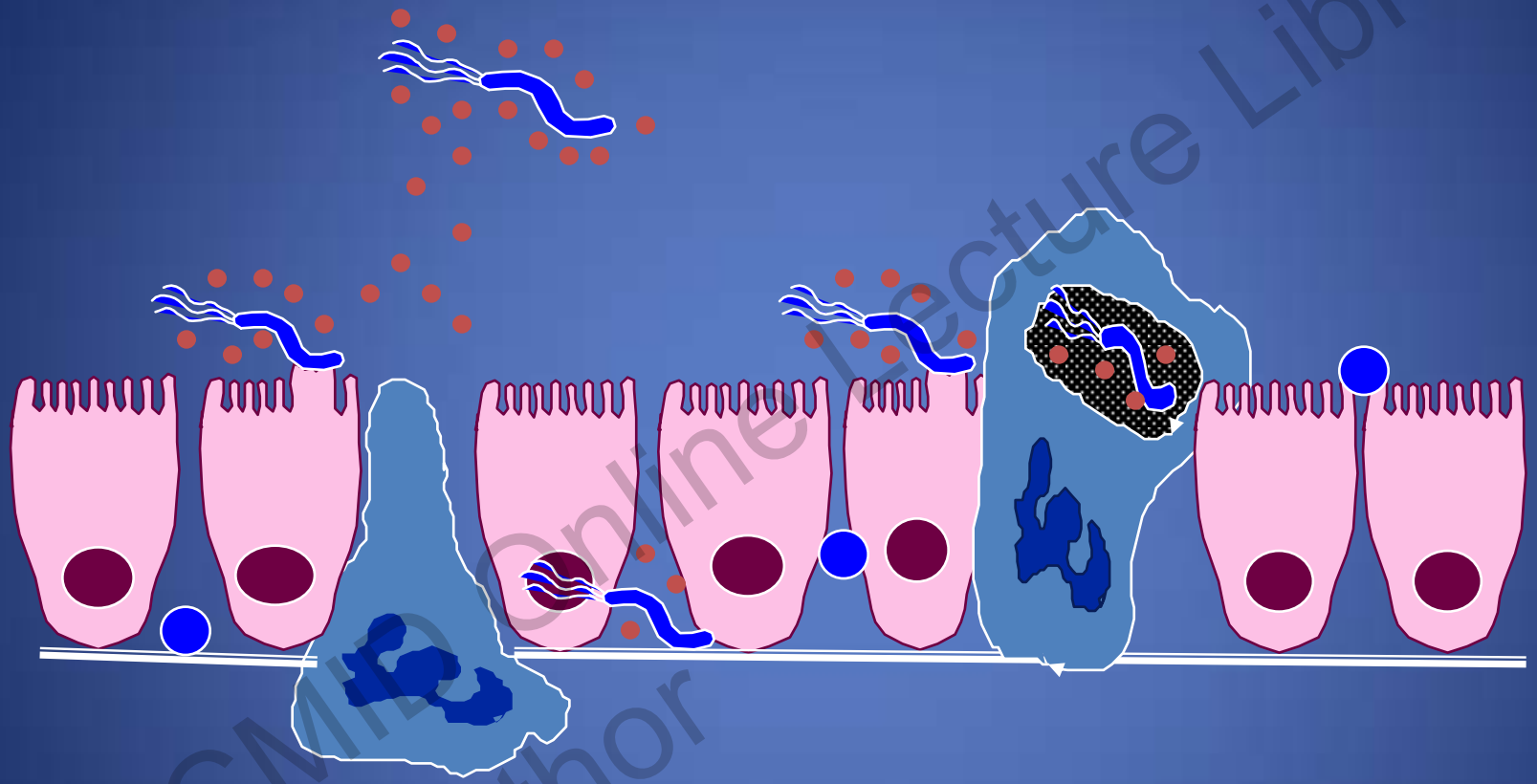
No of patiernts	Culture	PCR	Histology
1035	321 (31%)	474 (46%)	134/590 (23%)

In all studies there are a higher recovery rate of *H. pylori* by PCR than by culture no matter which primers (*ure*A, *ure*C, *hsp*60, *hp*16s) are used.

Difficult cultivable *H. pylori*.

- Fecal samples
- Dental samples
- Hepatic samples
- Gall bladder samples
- Environmental samples
- Water samples

Difficult-culturable *H. pylori*.



- ***H. pylori*** **may transform to coccoid forms in gastric mucosa and in environment.**

Difficult cultivable non-pylori *Helicobacter*?

- **Non-pylori *Helicobacter***
  - ***H. suis***
  - ***H. heilmannii***
  - ***H. pullorum***
  - ***H. valdiviensis***
  - ***etc.***

Real-time PCR.

- Rapid and quantitative.
- Selection of primers:
  - 23s rRNA also include clarithromycin resistance.
  - 16s rRNA also include tetracycline resistance.
  - Other primers only detect *H. pylori*.

Real-time PCR.

- **Eleven studies have compared culture and real-time PCR.**

No of patients	Culture	PCR	RT-PCR
1358	594 (44%)	188/506 (37%)	791 (58%)

- **The recovery rate of *H. pylori* is higher with RT-PCR than with conventional PCR, which is higher than for culture.**

Why is the recovery rate higher with PCR than with culture.

- Like histology, the method do not discriminate between live or dead *H. pylori*.
- Areas with small numbers and/or difficult culturable *H. pylori*.
- Occurrence of non-pylori *Helicobacter*.
- No subjective evaluation.
- Unspecific reactions.

Where have PCR for *H. pylori* diagnosis been used?

- **General diagnostics:**
  - **Gastric biopsies**
  - **Stool samples**

- **Where small amounts of *H. pylori* is expected:**
  - **Gallbladder**
  - **Dental plaques / oral cavity**
  - **Blood vessels**

Genomic susceptibility testing.

- Real time PCR using
  - 23s rRNA - Detects mutants for clarithromycin resistance.
  - 16s rRNA - Detects mutants for tetracycline resistance.
- Resistance to clarithromycin is more important than resistance to tetracycline.

Advances in using RT-PCR.

- **Fast diagnosis**
- **Detects ”difficult to culture” *H. pylori***
- **Detects non-pylori *Helicobacter*.**
- **Fast susceptibility testing.**
- **Reliably results.**
- **Useful in laboratories where there are no culture for *H. pylori*.**

Disadvances in using RT-PCR

- Do not discriminate between living and dead bacteria.
- Susceptibility testing is limited.
- Destroies protein – reprocessing/reuse impossible.

Proposed work set-up.

- **Use genus specific primers – *Helicobacter* spp.**
- **Use *H. pylori* specific primers (23s rRNA) – *H. pylori* plus clarithromycin resistance.**
- **Genus specific positive, *H. pylori* negative specimens: use non-pylori *Helicobacter* specific primers.**

Thank you. – Questions?