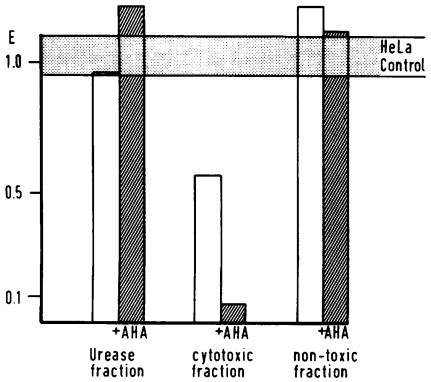

Effect of acetohydroxamic acid (AHA) on cytotoxic activity of H pylori: gel filtration fractions were added to HeLa cells either without or in the presence of 0·5 mg AHA/ml.

μm) supernatant fluids (1 mg protein/ml) of sonicates from various *H pylori* strains, obtained from endoscopy outpatients at the Department of Internal Medicine at the University Hospital Hamburg, were added in serial dilutions to 2×10^4 HeLa cells per well. After 48 hours of incubation photometric evaluation was carried out after staining residual cells with crystal violet. Results were expressed as CD50, defined as the highest titre of the sample leading to a 50% reduction of the optical density as compared with untreated cells. All 11 tested strains exhibited cytotoxic activity and yielded CD50s between 70 and 1867.

Gel filtration of sonicates using a sephacryl S 200 HR column (h = 100 cm, r = 0·8 cm; Pharmacia, Uppsala, Sweden) showed the cytotoxic fractions to be clearly distinct from the urease containing fractions.[6] To gain further evidence for the independence of this cytotoxic effect from the urease activity, we used AHA (Sigma, St Louis, Missouri, USA) as a urease inhibitor. At a concentration of 0·5 mg/ml, we found the *H pylori* urease activity to be effectively blocked (data not shown) as has been reported by Mobley *et al.*[1] While the addition of AHA to our cytotoxicity assay at the same concentration did not have an adverse effect on the HeLa cells when given either alone (control) or in combination with the urease fraction or a non-toxic fraction, combination of AHA with the cytotoxic fraction led to a sharp increase of cytotoxic activity (figure).

Thus AHA and some *H pylori* cytotoxic factors apparently act synergistically on HeLa cells. So far, we do not know the basis for this synergism. Tentatively, one might speculate that *H pylori* cytotoxin damages HeLa cell membranes, thus enabling AHA to accumulate intracellularly and then exert a cytotoxic effect. That hydroxamic acids actually have a cytotoxic potential in their own right on mammalian cells has been sufficiently demonstrated.[7,8] In view of these findings we would therefore advise refraining from any clinical trials incorporating AHA into therapeutic regimens aimed at healing *H pylori* induced gastritis, or even peptic ulcers, without further investigation of this synergistic effect.

H VON WULFFEN
T MARQUARDT
Institut für Medizinische Mikrobiologie und Immunologie, Universitätskrankenhaus Eppendorf, 20 246 Hamburg, Germany.

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Dr Allardyce and Mr Bagshaw comment:

We agree with Hinrik von Wulffen and Thies Marquardt that clinical trials should be undertaken with caution and in the light of all relevant in vitro and in vivo experimental evidence. It is also true that the publications on *H pylori* are littered with in vitro antibiotic data (including our own)[1] that may bear little relation to clinical outcome to the in vivo antibiotic functional efficacy in vivo.[2] There are, however, a number of points regarding results obtained using AHA in conjunction with *H pylori* sonicates on HeLa cells in vitro. First, the results from Hamburg do not include dose or time response data. The synergistic antimicrobial effects reported by us were obtained using five to 10-fold less AHA. Also, the mean effects of continuous 48 hour exposure of HeLa cells to *H pylori* sonicate fractions and AHA in culture are not accompanied by any indication of the variability of the observation or the length of time required for cellular damage to become irreparable. The issues of dose and timing are germane to the clinical finding that a single 750 mg dose of AHA inhibits *H pylori* urease in infected patients by 86%.[3] Second, there is a question relating to the practicality of the in vitro model using *H pylori* sonicates on HeLa cells as opposed to intact organisms on gastric epithelial cells (preferably mucus secreting) and the absence of control sonicate sephacryl fractions from organisms other than *H pylori*.

In the end, the clinical value of including urease inhibitors will rest on the balance between therapeutic efficacy and undesirable side effects. In vitro experiments, such as our own and those of von Wulffen and Marquardt, have some value by indicating general therapeutic directions and constraints. But that is all they can do. At present, what is needed are in vivo *Helicobacter* infection model experiments aimed at finding the most efficient means of eradicating *H pylori* at the least cost to the wellbeing of the host.

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Alternative method for transporting and storing gastric biopsy cultures of helicobacter pylori

Veenendal *et al* reported the use of sterile saline as a transport medium for gastric biopsy specimens in order to obtain adequate culture results for *Helicobacter pylori*.[1] Indeed, sterile saline is a simple and useful transport medium. Gastric biopsy specimens can, however, be put directly on to the surface of a suitable agar plate, thus avoiding the need for transport medium. This reduces costs, handling, and risk of contamination, which is the main problem in culturing *H pylori*.

In our hospital the microbiology laboratory supplies the endoscopy unit with agar plates suitable for *H pylori* culture. These are stored at 4°C in an ordinary freezer. Usually, our endoscopists take two gastric biopsy specimens with a sterilised forceps, and put them directly on to the surface of a suitable agar plate using a simple sterile needle, if necessary. The plate is subsequently stored in the freezer at 4°C until it is transferred to the microbiology laboratory. In our experience over 10 months, the specimens stored on agar plates may remain suitable for culture for at least seven days at 4°C (from seven to 14 days), and for at least 25 hours at room temperature (from 25 to 28 hours). Samples can therefore be collected, even from distant endoscopy units once or twice a week.

According to Veenendal *et al*, one antral biopsy specimen is adequate for culture. In our experience this holds true except in cases of previous omeprazole treatment, or where there is a strong reduction of parietal cells. In these circumstances *H pylori* may be present only in the oxyntic mucosa.[2,3] This may be due to the change in antral pH from acid to neutral, whereas a scanty local excretion of acid still present in the fundic area may permit *H pylori* survival in this zone.[2,4,5] A false negative culture of a single antral specimen may also be due to an extensive intestinal metaplasia of the antral mucosa.[6]

A SAVIO
Microbiology and Gastrointestinal Pathology Unit, S Orsola Fatebenefratelli Hospital, 25100 Brescia, Italy

1 Veenendal R A, Lichtendahl-Bernards A T, Pena A S, *et al.* Effect of transport medium and transportation time on culture of Helicobacter pylori from gastric biopsy specimens. *J Clin Pathol* 1993;46:561–3.
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Drs Veenendaal and Lichtendahl-Bernards comment:

Dr Savio raises two points. The first is an alternative method of transportation, processing, and storage of gastric biopsy cultures for *H pylori*. In our article we describe a low budget method of transporting gastric biopsy samples, without the need for refrigeration or a specialised transport medium, to a laboratory facility capable of culturing *H pylori*.

Although we are surprised and impressed by the long delay without loss of viability of the culture method described by Dr Savio, we feel that this method is more tailored to a situation in which facilities (refrigeration, culture media, a microbiology laboratory) are present in the same institution. In our experience, contamination of culture plates in an endoscopy department does occur and can be a problem (especially with yeasts) when culturing a fastidious organism like *H pylori*.

The second comment addresses the important point of how to detect or better exclude the presence of *H pylori* after treatment with drugs (omeprazole, bismuth, and several antibiotics) which influence the number and viability of the bacterium (coccoid forms). As most methods for detecting *H pylori* require a certain number of viable bacteria (histology, culture, and breath tests) to detect the bacterium, false negative test results are bound to occur after treatment. This problem is probably not solved by taking more specimens, and is at this moment the subject of further investigation.

In previously untreated patients we found (unpublished data) no positive culture results for *H pylori* from the gastric body when the gastric antrum was also not infected. In about 20% of our patients no inflammation or infection can be demonstrated in the gastric body region, which does not support the taking of additional body biopsy specimen for routine culture in previously untreated patients.

Clinical usefulness of detecting growth of *Mycobacterium tuberculosis* in positive Bactec phials using PCR

It has recently been shown that the polymerase chain reaction (PCR) can confirm growth of *Mycobacterium tuberculosis* in Bactec phials four to five days earlier than the use of DNA probes and seven to 10 days before presumptive identification by the Nomina Anatomica Parisiensia (NAP) growth inhibition test.[1] It has been suggested that a prospective evaluation of these methods is required.[1] We have investigated the PCR method to see if the earlier results provided would be of help in patient management.

Bactec 12B Phials are tested each morning. Those with a growth index between 20 and 50 are then read daily until the growth index falls or reaches 50, at which time a smear is made and a subculture performed. The smear and subculture plates are read the following day with updated reports being sent to the clinician when acid fast bacilli are detected. Confirmed or presumptive identity is reported as soon as colony morphology, NAP growth inhibition, or DNA probe results allow. We do not use smear morphology of positive phials to generate preliminary reports to physicians.[2]

For the three months March 1993 to May 1993, we stored 1 ml of fluid from all Bactec phials with a growth index of $\geqslant 20$. The aliquots were stored in centrifuge tubes at -70°C. For PCR, the aliquot was thawed and spun at 12 000 rpm for 10 minutes. The pellet was resuspended in 100 μl of a 10% chelex and 1% triton solution, sonicated for 15 minutes, and heated sequentially for 15 minutes each at 50°C and 95°C. Debris was pelleted at 12 000 rpm for two minutes and 2 μl of supernatant fluid was used for PCR according to previously published methods.[3] The PCR takes several hours to perform, after which it takes 90 minutes to run the gel. If PCR results from positive trials were clinically useful, we thought it might be possible to organise workflow so that the PCR result from a positive phial would be available the afternoon the phial became positive. We therefore calculated the time advantage for PCR as if the PCR result was available the afternoon the phial became positive.

Aliquots were stored from 247 phials: 24 contained *M tuberculosis*; 48 contained other myocbacteria; 74 contained bacteria only; and 87 were sterile. The 24 specimens containing *M tuberculosis* came from 10 patients. Fifteen of the 24 (72%) original specimens containing *M tuberculosis* were smear positive, and five of the 10 patients had specimens with positive smears. Aliquots from 86 phials were subjected to PCR: all 24 containing *M tuberculosis*, none of which contained bacteria; all 48 containing other mycobacteria, seven of which contained bacteria; and 14 which did not contain mycobacteria, 10 of which contained bacteria. All phials containing *M tuberculosis* were correctly identified, with a growth index as low as 21 (mean 266, range 21–999) including 12 phials with a growth index of <100, three of which were smear negative. It took an average of 16 days, range 6–45 days, for the phials containing *M tuberculosis* to give a positive growth index. Recovery of *M tuberculosis* could have been confirmed by PCR five days (range one to 13) before presumptive or confirmed growth of *M tuberculosis* was made by other methods. No false positive PCR results were observed among the other 62 phials analysed. Although multiple bands were observed on the gel from one phial containing *M chelonae*, no band was positive on specific probing.

The clinical utility of the PCR result was assessed by examining the medical records of all 10 patients infected with *M tuberculosis* to determine whether the result would have enabled earlier treatment or aided in infection control measures. Nine of the 10 patients were already receiving treatment for 17 days (range three to 50) before the Bactec phial turned positive. All five patients with specimens with positive smears were either receiving treatment at the time or started treatment when the smear result became positive. Four patients with smear negative specimens were already receiving treatment by the time the Bactec phial became positive. Only one patient with a smear negative, culture positive sputum specimen, who was discharged the same day the phial became positive, may have benefited from the PCR result. As it was, this patient was promptly recalled and treatment initiated on receipt of the phial smear result. While PCR of positive phials would have had no clinical impact, a positive PCR result on the original specimen would have been helpful for two of the five smear negative patients: they would have had treatment 10 to 13 days earlier.

PCR is beginning to be compared with contemporary culture methods in large scale studies.[4] The sensitivity is high for smear positive specimens, but is considerably lower for smear negative ones—94% *v* 62%, respectively.[4] Although this type of evaluation is a useful first step, we should not be misled into believing that it is necessarily going to improve dramatically clinical management. For most smear positive patients, the result may not change what is done. It would be most useful for smear negative patients if the result led to earlier treatment, but even in this situation, as shown in this study, many patients are appropriately being given treatment based on clinical and family history, physical and radiological findings, and skin test results. In this study the phial PCR result was 100% sensitive and specific. A positive PCR result therefore removes the need to use either the DNA probe for *M tuberculosis* or the NAP test. We agree with the suggestion of Cormican *et al* that this methodology requires prospective evaluation against contemporary diagnostic methods.[1] Such comparisons should take into account technologist time and workflow benefit for the mycobacteriology laboratory.

A MORRIS
L RELLER
Clinical Microbiology Laboratory
B DEVLIN
Molecular Diagnostic Laboratory, Duke University Medical Center, Durham, NC 27710, USA

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Dr Cormican and colleagues comment:

We read with interest the comments of Morris and colleagues on the use of PCR to confirm the presence of *Mycobacterium tuberculosis* in positive Bactec phials and are pleased that they confirm the essence of our original report. We agree with the authors that there are two issues to consider in relation to the application of PCR based methods in the clinical laboratory.

In relation to clinical practice, we agree that the savings in time achieved by PCR relative to conventional methods may be expected to benefit a relatively small group of patients with tuberculosis in whom there is an urgent need for diagnosis and real clinical uncertainty following application of conventional methods. The benefit to