

Peptest for diagnosing gastro-oesophageal reflux

Medtech innovation briefing

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Summary

The Peptest is designed to help diagnose gastro-oesophageal reflux from the stomach into the oesophagus, larynx or airways, by detecting pepsin within a saliva or sputum sample. Currently, there is no agreed normal range for pepsin levels in saliva. Three fully published small studies compared the Peptest with a reference standard. Two of these studies reported sensitivity of 33–78% and specificity of 100–43%. Each Peptest costs £10–20 depending on where it is processed and whether a quantitative or qualitative result is needed.

Likely place in therapy	Effectiveness and safety
<ul style="list-style-type: none"> The Peptest is a non-invasive, near patient test to help diagnose gastro-oesophageal reflux disease (GORD) including extra-oesophageal reflux, by detecting pepsin in saliva or sputum. Most patients are diagnosed with GORD based on their symptoms alone. A salivary test for pepsin could be used in patients who remain symptomatic after empirical acid suppressing treatment, or who have atypical ear, nose and throat symptoms of GORD. 	<ul style="list-style-type: none"> One cross-sectional study (Ocak et al. 2015) evaluated the accuracy of pepsin detection in saliva using the Peptest in 20 people with suspected laryngopharyngeal reflux. The Peptest was reported to have a sensitivity of 33% and a specificity of 100% compared with pH monitoring. One prospective controlled cohort study (Hayat et al. 2015) tested the Peptest's ability to discriminate between people with GORD (n=58), people with hypersensitive oesophagus (n=26) and people with functional heartburn (n=27). The diagnostic accuracy of Peptest compared with pH MII monitoring for GORD was not reported, but equates to a sensitivity of 78% and specificity of 62%. One case-control study (Hayat et al. 2014) used the Peptest to measure the pharynx's exposure to pepsin in patients with reflux-related hoarseness (n=21) and people with no symptoms (n=10). Each patient gave 5 saliva samples over 24 hours. The symptomatic group had 28 positive samples from 13 of 21 patients, compared with 6 positive samples from 4 of 10 asymptomatic patients. The samples from symptomatic patients were more likely to test positive than those from the control group (26.7% compared with 12%, p=0.025). Overall, there is limited published evidence on the diagnostic accuracy of the Peptest for GORD and laryngopharyngeal reflux, and it is uncertain how generalisable it is to the test's likely place in therapy.

Technical factors	Cost and resource use
<ul style="list-style-type: none"> • Patient-collected samples can be either processed locally, if suitable laboratory equipment is available for sample preparation, or posted to the manufacturer, RD Biomed, for testing in its laboratory. • A bench-top vortex mixer and micro-centrifuge are needed for processing samples locally, and will give a qualitative result. An electronic lateral flow device reader is also needed to give a quantitative result. • The Peptest has a limit of pepsin detection of 16 ng/ml. There is no agreed normal range for pepsin levels in saliva. • After sample processing, qualitative results are ready in 15 minutes. 	<ul style="list-style-type: none"> • Each Peptest costs £10 to £17 for local testing. • An electronic lateral flow device reader (for quantitative analysis) costs £1100 (excluding VAT). • For samples sent to the manufacturer's laboratory, testing costs £41.66 for 2 samples or £60.00 for 3 samples. • Because pepsin concentration varies throughout the day, the manufacturer recommends that 3 samples are taken over 1–2 days to confirm diagnosis.

Introduction

Gastro-oesophageal reflux disease (GORD), also known as acid reflux, is a chronic condition in which the contents of the stomach regurgitate into the oesophagus or larynx. This happens when the lower oesophageal sphincter, the valve between the stomach and oesophagus, is weak or relaxes inappropriately.

The regurgitated liquid usually contains stomach acid and the stomach enzyme pepsin. Unlike the mucosal cells that line the stomach, the mucosal cells that line the oesophagus, larynx and airways are not resistant to acid. Therefore, the stomach acid can inflame and damage the lining of the oesophagus causing oesophagitis, heartburn, sore throat and dysphagia. In the larynx it can cause sore throat and voice disorders. The severity of the symptoms depends on the degree of sphincter dysfunction, the type and amount of fluid brought up from the stomach, and the neutralising effect of saliva.

According to NICE guidance on [dyspepsia](#), GORD is a common condition. About 1 in 5 people are thought to experience at least 1 episode of GORD per week, with 1 in 10 people experiencing symptoms of GORD daily. People of all ages can be affected, including children, but it is more prevalent in adults aged 40 years or older. Severe cases of oesophagitis can cause the formation of oesophageal ulcers that may bleed, causing pain and making swallowing difficult. Repeated episodes of GORD can lead to changes in the cells in the lining of the lower oesophagus, a condition known as Barrett's oesophagus. This condition is estimated to affect 1 in 10 people with GORD. Barrett's oesophagus is characterised by pre-cancerous changes to the cells lining the oesophagus. These cells have an increased risk of becoming cancerous in time. Conversion of Barrett's oesophagus to oesophageal adenocarcinoma has a lifetime risk of 5% in men and 3% in women (Jankowski 2010).

GORD is normally diagnosed empirically with a trial of proton pump inhibitors or by endoscopy, manometry or pH testing when more serious disease is suspected. It may present with atypical symptoms, including chronic cough, hoarseness, loss of voice, laryngeal pain or ear, and nose and throat symptoms caused by stomach contents reaching the larynx and trachea. This is known as laryngopharyngeal reflux.

Although the presence and quantity of pepsin in saliva may indicate the presence of GORD, there is no consensus in the published literature about what concentration of pepsin would be considered clinically relevant. Published studies have used different pepsin concentrations ranging from 16 ng/ml to 25 ng/ml as a cut-off value to indicate a clinically significant concentration of pepsin in saliva.

Technology overview

This briefing describes the regulated use of the technology for the indication specified, in the setting described, and with any other specific equipment referred to. It is the responsibility of healthcare professionals to check the regulatory status of any intended

use of the technology in other indications and settings.

About the technology

CE marking

The Peptest is classified as an in-vitro diagnostic medical device, general classification. The manufacturer, RD Biomed, received a CE mark for the Peptest in January 2011.

Description

The Peptest is a non-invasive, single-use, in-vitro diagnostic medical device for detecting pepsin in saliva or sputum. Samples are collected by patients at home in sample tubes, which contain citric acid as a preservative, and can be posted to RD Biomed's laboratories for analysis or processed locally if suitable equipment is available (see costs section for details of test components).

The Peptest is a lateral flow device that contains 2 types of unique proprietary monoclonal antibodies against pepsin, on a nitrocellulose membrane in a plastic case. One antibody is tagged with a colorimetric marker and becomes soluble on application of a test sample. The other antibody is immobilised onto the nitrocellulose membrane, at the test line. Any pepsin in the sample is bound at the test line by the 2 antibodies, and a coloured line is seen through the viewing window in the plastic case.

If the sample is processed locally, a bench-top vortex mixer and micro-centrifuge are needed. The sample is centrifuged to remove any insoluble material and mixed with a migration buffer. This diluted sample is then applied to the sample well of the Peptest device. Any pepsin in the sample is bound at the test line by the 2 antibodies and, after 5 to 15 minutes, a coloured line becomes visible through the viewing window in the plastic case, giving a qualitative result. The test line is visible if Pepsin is present at a concentration of 16 ng/ml or more.

A quantitative test result can be obtained by analysing the intensity of the test line using an electronic lateral flow reader. There is currently no consensus in the literature around the concentration of pepsin in saliva that is clinically relevant, and so the value of a quantitative result in guiding clinical management is unclear. The level of pepsin can be quantified if it is present at a concentration of 25 ng/ml or more.

Intended use

The Peptest is designed for use where diagnosing GORD using a saliva sample or laryngopharyngeal reflux using a sputum sample would be advantageous, and where other diagnostic tests are not available, not easily accessed, or not appropriate for a specific patient.

Setting and intended user

The Peptest can be used in primary or secondary care. Samples can be posted to RD Biomed for analysis or processed locally where the necessary laboratory equipment is available. People can also buy the Peptest directly from RD Biomed but this use is outside of the scope of this briefing,

Reflux levels may vary over the course of a day. The manufacturer recommends that 3 samples are taken over 1–2 days to confirm diagnosis.

If the samples are being processed locally, such as in a hospital laboratory, a vortex mixer and a bench-top centrifuge (and staff trained in their use) must be available. For quantitative analysis of the test results, an electronic lateral flow device reader is also needed. Samples sent to RD Biomed's laboratory by GPs or hospital doctors are processed, tested and quantified, and a report is sent to the patient and doctor.

Current NHS options

NICE guidance on [dyspepsia](#) makes recommendations on the diagnosis, management and treatment of GORD. The aims are to control symptoms, heal oesophagitis and prevent recurrent oesophagitis or other complications.

The guideline recommends referral for endoscopy if there are any red-flag symptoms, which include:

- gastrointestinal bleeding
- persistent vomiting
- progressive unintentional weight loss
- aged 55 years or older and initial acid suppression treatment has failed.

Endoscopy is used to view the oesophagus and take biopsies. The oesophagus is examined for any oesophageal mucosal breaks, which are used to grade any oesophagitis. Oesophageal mucosal breaks are clearly demarcated areas of slough or erythema along the mucosa of the oesophagus.

If a person has GORD but no red-flag symptoms, no confirmatory tests are done. Treatment is based on advice on lifestyle changes and a medication review, because some drugs such as non-steroidal anti-inflammatories can cause GORD. If the symptoms remain uncontrolled then proton pump inhibitors may be offered to control gastric acid secretion. If this is successful, then a step-down approach is used to lower the dose or return to self-care. A test for *Helicobacter pylori* is also offered at this stage in the patient pathway. If symptoms continue despite proton pump inhibitor treatment, further investigations may be necessary.

The British Society for Gastroenterology Guidelines for Oesophageal Manometry and pH Monitoring (Bodger and Trudgill 2006) state that flexible endoscopy or contrast radiology (such as barium swallow) should be used for people with suspected oesophageal symptoms, and if these tests are not conclusive then oesophageal manometry should be considered. Oesophageal manometry is used to evaluate dysphagia, and to diagnose primary oesophageal motility disorders (achalasia and diffuse oesophageal spasm). Manometry is carried out via a naso-gastric tube and assesses how well the lower oesophageal sphincter is working by measuring pressure levels inside the sphincter muscle. Oesophageal pH monitoring is also done using a naso-gastric tube over 24 hours. Manometry can rule out oesophageal motility disorders and pH monitoring can confirm acid reflux.

Oesophageal pH monitoring may be used to investigate:

- oesophageal motility disorders
- where there has been no response to proton pump inhibitors
- chest pain
- throat or respiratory symptoms or before anti-reflux surgery
- when symptoms persist after surgery.

During this procedure, pH electrodes for ambulatory oesophageal 24-hour pH monitoring are placed in the oesophagus, and the patient wears a data recorder to record

symptomatic episodes of reflux. Data can be analysed for any correlation of decreased oesophageal pH with episodes of reflux.

In general, manometry and oesophageal pH monitoring are relatively uncommon investigations which tend to be done only in specialist gastroenterology settings.

These symptoms are non-specific and there is no reference test for such 'extra-oesophageal reflux'. If GORD is suspected in chronic cough, patients can be treated in the same way as for typical presentations (Irwin, 2006).

GORD also occurs in children. However the NICE guideline on [gastro-oesophageal reflux \(GOR\) in children](#) highlights the difference between GOR, which is a normal physiological process associated with eating, and GORD, for which medical attention should be sought. The guideline describes the further investigations needed if GOR is present with 'red flags' and recommendations for treating symptomatic GORD that is causing distress or pain. Although GORD in children and adolescents often arises in similar ways to GORD in adults, premature babies and children with complex and severe neuro-disabilities are at increased risk of GORD.

In children, an oesophageal pH study (or combined oesophageal pH and impedance monitoring if available) should be considered. This can be used to confirm the GORD diagnosis where there is:

- suspected recurrent aspiration pneumonia
- unexplained apnoeas
- unexplained non-epileptic seizure-like events
- unexplained upper airway inflammation
- dental erosion associated with a neurodisability
- frequent otitis media
- a suspected diagnosis of Sandifer's syndrome
- a possible need for fundoplication (a surgical procedure) to manage severe GORD.

In these less common presentations, a definitive rather than symptomatic diagnosis is needed.

Recognising GORD in infants, children and young people with a severe neuro-disability who have limited ability to communicate presents a particular challenge.

NICE is not aware of other CE-marked devices that have a similar function to the Peptest.

Costs and use of the technology

The manufacturer provides a number of options for purchasing the Peptest depending on individual needs (all prices are excluding VAT).

Costs of near-patient or local testing

A kit of 10 Peptest devices with associated equipment and consumables costs £170. Each kit includes:

- 10 Peptests
- 10 graduated pipettes
- 10 collection tubes containing citric acid
- 20 dual bulb pipettes
- 10 micro-tubes of migration buffer
- 10 micro-centrifuge tubes.

A kit of 50 Peptest devices with sufficient consumables costs £500.

Additional items are available from the manufacturer:

- An electronic lateral flow device reader is needed for quantitative analysis: cost £1100
- 50 Peptest collection tubes containing citric acid: cost £37
- 125 ml migration buffer (sufficient for 500 tests): cost £20
- 125 ml citric acid (sufficient for 250 tests): cost £20.

The lateral flow device reader is under warranty for 12 months and has an expected lifetime of 3–5 years depending on the level of use. A reference device is provided with

each reader that allows for calibration every 250 readings. The manufacturer is planning to make an annual service contract available for the lateral flow device reader, but the planned costs are currently not available. RD Biomed provides full training in the use of the reader, free of charge.

Samples may be processed locally (both a vortex mixer and a centrifuge are needed) or by RD Biomed at its central laboratory.

Costs of performing tests at a central laboratory

For Peptest analyses conducted at RD Biomed's laboratory, 2 samples cost £41.66 and 3 samples cost £60. This price includes sample tubes, instructions, and postage and packaging. The samples are tested before the results are quantified and results sent back to the doctor. As the level of pepsin in saliva varies between people and over the course of the day, the manufacturer recommends that 3 saliva samples are collected over the course of 1–2 days.

Likely place in therapy

In most cases, GORD is diagnosed through patient-reported symptoms alone without any tests being done. Further investigation is needed only if the patient has dysphagia (an inability to swallow), or if medication and lifestyle changes have not improved symptoms. The Peptest would therefore not be used on all patients who present with GORD symptoms, but could be used when a non-invasive diagnosis of reflux is needed. For example, a positive Peptest result after the failure of first-line treatment may confirm a diagnosis of GORD, without needing further invasive diagnostic tests such as endoscopy. Peptest could also be used as an additional test in patients with ear, nose and throat symptoms where GORD is suspected. It could be used in patients with atypical symptoms that have not responded to lifestyle changes or treatment with proton pump inhibitors.

The manufacturer's instructions for use state that test results should be evaluated in conjunction with other clinical data available.

Specialist commentator comments

Two specialist commentators indicated that the role of the Peptest is limited, because most patients would not need an endoscopy and the dyspepsia guidelines recommend

treatment for symptoms without investigation.

One commentator noted that the cut-off for a positive result varied in the published studies and that considerable further study would be needed to confirm the chosen limit. Another commentator noted that none of the published studies involved children, and felt that a study in children would be helpful.

One specialist commentator stated that the Peptest may have a role in unusual presentations of GORD, where there are no alarm symptoms to prompt referral to endoscopy. These would include cases of chronic cough, chronic sore throat, hoarseness or unexplained chest pain. A positive Peptest early in the diagnostic process could save resources spent on unnecessary investigations, subsequent scans and tests. The commentator added that Peptest does not indicate the cause of GORD, and so it would still be important to consider and rule-out serious potential causes for these symptoms.

One commentator noted that the drugs used to treat GORD are low cost and effective and so in most cases the Peptest would not be useful. A second commentator agreed, stating that prescribing proton pump inhibitors for a trial period is an effective way to confirm reflux. They added that although the test could be useful for people whose GORD does not respond to proton pump inhibitors, there is currently no treatment to offer except surgery. In order to use Peptest as a basis for recommending surgery, sizeable clinical trials would be necessary to prove any clinical benefit.

Equality considerations

NICE is committed to promoting equality and eliminating unlawful discrimination. We aim to comply fully with all legal obligations to:

- promote race and disability equality and equality of opportunity between men and women
- eliminate unlawful discrimination on grounds of race, disability, age, sex, gender reassignment, pregnancy and maternity (including women post-delivery), sexual orientation, and religion or belief, in the way we produce our guidance (these are protected characteristics under the Equality Act 2010).

NICE guidance on [gastro-oesophageal reflux \(GOR\) in children](#) has identified that research is needed to identify which symptoms of reflux are indicative of GORD in children with

severe neuro-disability at risk of GORD. These children may not be able to communicate their symptoms as a result of their disability. GORD is more prevalent in people aged over 40 years. Age and disability are protected characteristics under the 2010 Equalities Act.

Evidence review

Clinical and technical evidence

Regulatory bodies

A search of the Medicines and Healthcare products Regulatory Agency (MHRA) website revealed no manufacturer Field Safety Notices or Medical Device Alerts for this equipment.

No reports of adverse events were identified from searches of the Medicines and Healthcare products Regulatory Agency (MHRA) website, or from the US Food and Drug Administration database: Manufacturer and User Facility Device Experience (MAUDE).

Clinical evidence

Four fully published studies of the Peptest (Yuksel et al. 2012, Ocak et al. 2015, Hayat et al. 2015; Hayat et al. 2014) and 3 studies published as abstracts (Hayat et al. 2013, de Bortoli et al. 2012, de Bortoli et al. 2013) were identified to be relevant to this briefing. One of the fully published studies (Yuksel et al. 2012) was excluded because the manufacturer stated that it used an early prototype version of the Peptest and not the commercially available test. All of the fully published studies included adults only.

The study by Ocak et al. (2015, table 1) was a cross-sectional study in Turkey, evaluating the accuracy of pepsin detection in saliva using the Peptest. The trial enrolled 20 patients with suspected laryngopharyngeal reflux (LPR) but the method of recruitment was not clear. All 20 had 24-hour oesophageal pH monitoring. Each patient also gave a single sputum sample at the point in the 24 hours when they had their worst symptoms. In this study, the term 'pathologic gastro-oesophageal reflux' was used to define the threshold at which gastro-oesophageal reflux causes GORD.

Pathologic gastro-oesophageal reflux was defined as the distal pH probe detecting pH of less than 4 for over 5% of the 24 hours. The Peptest results showed sensitivity of 33%,

specificity of 100%, positive predictive value (PPV) of 100% and negative predictive value (NPV) of 14.2% for diagnosing pathologic gastro-oesophageal reflux. The test accuracy of the Peptest for diagnosing LPR was not reported.

The UK-based study by Hayat et al. (2015, table 2) had 2 aims: firstly, to establish normal values of salivary pepsin using the Peptest in healthy asymptomatic people, and secondly to determine the Peptest's ability to discriminate between people with reflux-related symptoms, including GORD and hypersensitive oesophagus, and those with functional heartburn. A cut-off value of 16 ng/ml pepsin in saliva was used to decide a positive test result for pepsin. Data were analysed from 111 patients with typical GORD symptoms (predominant heartburn with or without regurgitation) and 100 asymptomatic healthy volunteers. Both groups completed impedance-pH (MII-pH) monitoring and simultaneous salivary pepsin test on 3 samples during 24 hours. Outcome measures included prevalence of positive pepsin detection in saliva, pepsin concentration in saliva, timing of positive pepsin samples, correlation between pepsin in saliva and reflux parameters.

The study reported that, of the 111 symptomatic patients, 58 were objectively classified as having GORD, based on having an increased oesophageal acid exposure time greater than 4.2%, based on MII-pH. The remaining 53 people had a normal oesophageal acid exposure time and were classified as either having hypersensitive oesophagus (n=26) or functional heartburn (n=27). Of the 58 objectively classified GORD patients, 45 had at least 1 positive test for pepsin out of the 3 samples taken. Twenty-one of the patients classified as having hypersensitive oesophagus and 9 of those classified as having functional heartburn had at least 1 positive test for pepsin out of the 3 samples. Notably, 33 of the healthy, asymptomatic people had at least 1 positive test for pepsin out of 3 samples.

Hayat et al. (2014, table 3) aimed to quantify pharyngeal exposure to gastric contents using a number of new diagnostic techniques in patients with reflux-related hoarseness and healthy people. The patient population in this study is therefore different from patients with GORD who do not have reflux-related hoarseness. The study was conducted in the UK. It included 21 patients with hoarseness, who had been diagnosed with laryngopharyngeal reflux on the basis of clinical evaluation (questionnaires) and laryngoscopy, and 10 patients with no symptoms of GORD or voice disorders. Patients provided 5 saliva samples throughout the day to be analysed by the Peptest. The presence or absence of pepsin in the saliva was determined with a cut-off value of 25 ng/ml.

The study found that there were 28 positive pepsin samples from 13 of the 21 patients

with hoarseness (62%), and 6 positive samples from 4 of the 10 patients with no symptoms (40%). Only 1 of the 10 patients with no symptoms had 2 or more positive samples, compared with 9 of 21 with hoarseness.

Three studies that were published as abstracts were identified as being relevant to this briefing (Hayat et al. 2013, de Bortoli et al. 2012, de Bortoli et al. 2013). Two abstracts (Strugala et al. 2007a, 2007b) were also identified but were excluded, because the manufacturer stated that the Peptest used in these 2 studies was an early pre-production, non-CE-marked prototype. The 3 relevant abstracts provided very limited information on aspects of the study settings, methods, characteristics and results. The studies are outlined in table 4.

Table 1 Summary of the Ocak et al. (2015) study

Study component	Description
Objectives/ hypotheses	To evaluate the accuracy of pepsin detection in the saliva using the Peptest for the diagnosis of LPR.
Study design	<p>Cross-sectional study.</p> <p>All patients had 24-hour oesophageal dual pH monitoring, during which each patient gave 1 sample of sputum Peptest when they had the worst symptoms.</p> <p>The Peptest was expected to have the ability to detect pepsin down to 16 ng/ml.</p> <p>Ambulatory pH monitoring and pepsin detection test analysis were double-blinded by separate researchers.</p>
Setting	<p>Unclear about the setting but the study was conducted in Turkey.</p> <p>Further details were not reported. No information was provided on the source of the patients, including how they were recruited.</p>

Inclusion/ exclusion criteria	Inclusion criteria: people with suspected LPR, who had at least 1 LPR symptom, with RSI>15 and RFS>3. Exclusion criteria: psychiatric disorders with cooperation disability; previous laryngeal surgery history; any kind of nasal, paranasal, pharyngeal, laryngeal or pulmonary disease which can mimic LPR symptoms; patients who had taken proton pump inhibitors in the last 1 month.
Primary outcomes	Pathologic GOR findings (percentage of time pH<4 in distal probe over 5%); LPR findings (presence of a single attack of pH<4 in the proximal probe); pH score in the proximal and distal probes when the sputum sample was given; sensitivity, specificity; PPV; NPV.
Statistical methods	Not reported.
Participants	People with a suspicion of LPR (n=20).

<p>Results</p>	<p>Mean RSI: 22.1</p> <p>Mean RFS: 8.1</p> <p>Patients with at least 1 LPR attack (pH<4 in the proximal probe): 90%.</p> <p>Mean pH value: 6.38 in the proximal probe and 4.32 in the distal probe at the sample time.</p> <p>Patients with pathologic GOR findings (percentage of time pH<4 in distal probe over 5%): 90%.</p> <p>All pepsin-positive patients were in the pathologic GOR group.</p> <p>For pepsin detection test for GOR:</p> <ul style="list-style-type: none"> • sensitivity 33% • specificity 100% • PPV 100% • NPV 14.2% <p>Among the 6 people with a pepsin positive test, pH<4 in 66% of the distal probe and 33% of the proximal probe at the sampling time.</p> <p>Pepsin-positive people had an apparent acidic pH value in the proximal probe at the sample time compared with the pepsin-negative patients (3.26 compared with 6.81).</p>
<p>Conclusions</p>	<p>The authors concluded that, because of the benefits and ease of application, a positive salivary pepsin test in a patient suspected of having LPR can be a cost effective, accurate and alternative diagnostic method. Increasing the daily number of sputum samples may increase the sensitivity of the test.</p>
<p>Abbreviations: GOR, gastro-oesophageal reflux; LPR, laryngo-pharyngeal reflux; n, number of patients; NPV, negative predictive value; PPV, positive predictive value; RFS, reflux finding score (details available in table 2 of the paper); RSI, reflux symptom index (0=no problem; 5=severe problem).</p>	

Table 2 Summary of the Hayat et al. (2015) study

Study component	Description
Objectives/ hypotheses	To establish normal values of salivary pepsin in healthy asymptomatic people and to determine its value to discriminate patients with reflux-related symptoms (GORD, HO) from functional heartburn, using Peptest.
Study design	<p>A prospective controlled cohort study. Patient sampling was consecutive.</p> <p>Cut-off pepsin concentration for a saliva sample positive for pepsin was >16 ng/ml. Each patient had 3 samples during the day: on waking, 1 hour after finishing lunch and 1 hour after finishing dinner during the 24-hour ambulatory MII-pH monitoring period.</p> <p>Analysis was blinded to the patients' status, any reflux monitoring parameter and Reflux Disease Questionnaire scores.</p>
Setting	Healthy volunteers were recruited by advertisements placed at St George's University of London, and consecutive patients with typical GORD symptoms were referred to the Upper Gastrointestinal Physiology Unit at the Royal London Hospital for reflux assessment.
Inclusion/ exclusion criteria	<p>Patients with typical GORD symptoms (predominant heartburn with or without regurgitation) or with a primary complaint of heartburn were included. Patients were excluded if they had a history of previous oesophageal/gastric surgery, or a known oesophageal motor disorder (e.g. achalasia, scleroderma).</p> <p>For the control: asymptomatic healthy volunteers.</p>
Primary outcomes	Prevalence of positive pepsin detection in saliva; pepsin concentration in saliva; timing of positive pepsin samples; correlation between pepsin in saliva and reflux parameters; pepsin concentration in saliva to differentiate patients with GORD, or patients with reflux-related symptom (GORD+HO) from patients with FH.

<p>Statistical methods</p>	<p>Data were expressed as mean±SEM or median (IQR) where appropriate. A p value of <0.05 was considered significant. A ROC curve was constructed to determine and compare the sensitivity and specificity of different pepsin cut-off concentrations and their predictive value to diagnose or refute the diagnosis of GORD and reflux-related symptoms.</p>
<p>Participants</p>	<ul style="list-style-type: none"> • Symptomatic group: 134 patients with typical GORD symptoms, 111 of them completed MII-pH monitoring and simultaneous salivary pepsin test on 3 samples and were included in the analyses. Of these 111 patients, 58 had increased (>4.2%) AET, and were classified as having GORD; the rest had normal AET and were classified as having HO (n=26, with SAP+) and FH (n=27, with SAP-) respectively^a. • Control group: 104 asymptomatic healthy volunteers, 100 of them completed MII-pH monitoring and simultaneous salivary pepsin determination on 3 samples. Of these 100 volunteers, 87 who had normal MII-pH were included in the analyses^b.

<p>Results^c</p>	<p>Of the 58 patients classified as having GORD, 45 had at least 1 positive test for pepsin out of the 3 samples. Twenty-one of the patients classified as HO and 9 of those classified as FH had at least 1 positive test for pepsin out of the 3 samples. Diagnostic accuracy of Peptest in these 111 symptomatic patients was not reported in the paper but can be calculated as follows (based on number of patients tested): sensitivity 78%, specificity 43%, PPV 60%, NNP 64%, LR for positive test of 1.37, and LR for negative test 1.94, when at least 1 out of 3 samples was positive.</p> <p>Of the 87 asymptomatic healthy people who had a normal MII-pH, 33 had at least 1 positive test for pepsin out of the 3 samples. Diagnostic accuracy of Peptest in the 58 patients objectively diagnosed with GORD comparing with these 100 healthy controls was not reported; it can be calculated as follows (based on number of patients tested): sensitivity 78%, specificity 62%, LR for positive test 2.05, LR for negative test 2.77, and false positive rate 38%, when at least 1 out of 3 samples was positive. However, this calculation presumes that all the controls had been confirmed to be truly negative by the same reference standard used for the patients.</p> <p>When measuring the highest pepsin concentration in saliva out of the 3 samples, 1 third of asymptomatic patients had pepsin in saliva at low concentration: median 0 ng/mL; 25–75th centiles 0–59; 95th centile 190.6.</p>
<p>Conclusions</p>	<p>In patients with symptoms suggestive of GORD, salivary pepsin testing may complement questionnaires to assist office-based diagnosis. This may lessen the use of unnecessary anti-reflux therapy and the need for further invasive and expensive diagnostic methods.</p>

Abbreviations: AET, (oesophageal) acid exposure time; ANOVA, analysis of variance; CI, confidence interval; FH, functional heartburn; GORD, gastro-oesophageal reflux disease; HO, hypersensitive oesophagus; ITT, intention to treat; IQR, inter-quartile range; LR, likelihood ratio; MII-pH, impedance-pH; NPV, negative predictive value; n, number of patients; PPV, positive predictive value; RR, relative risk; ROC, receiver operator characteristic curves; SEM, standard error of the mean.

^aSAP, symptom association probability, which was used to characterise the association between reflux and symptoms. No further details were described.

^bIt was unclear whether MII-pH monitoring result for this group was also evaluated based on the cut-off value of the oesophageal acid exposure time of 4.2%.

^cOnly outcomes that are relevant to this briefing report were reported in this table.

Table 3 Summary of the Hayat et al. (2014) study

Study component	Description
Objectives/ hypotheses	To assess new methods for the objective detection of oesophago-pharyngeal reflux and for quantification of pharyngeal exposure to gastric contents in patients with hoarseness and asymptomatic controls.
Study design	<p>Case-control study.</p> <p>Pepsin in the saliva sample was detected using Peptest. Each person had 5 saliva samples collected for the analysis of pepsin presence. The presence or absence of pepsin in the saliva was determined with a cut-off value of 25 ng/ml.</p> <p>It was unclear whether the individual who tested the samples and who interpreted the test were blinded to sample source and subject classification (GORD compared with healthy volunteers).</p>
Setting	Patients were recruited from a tertiary referral specialist voice clinic in the UK. No further details about the setting were reported, nor information about the source of healthy volunteers.

<p>Inclusion/ exclusion criteria</p>	<p>Patients with hoarseness who had been diagnosed with laryngo-pharyngeal reflux on the basis of clinical evaluation (questionnaires) and laryngoscopy were included. Controls were healthy adult volunteers with no symptoms of GORD or voice disorders (evaluated in the same way with identical questionnaires and laryngoscopy).</p> <p>People who smoke were excluded, along with patients with chronic cough, significant pulmonary or neuro-musculo-skeletal disease, or where voice misuse was suspected. Patients were excluded if they had a previous laryngeal surgery, history of oro-pharyngeal/laryngeal cancer, previous gastric or oesophageal surgery, or endotracheal intubation in the last 3 months. All patients were studied 'off' proton pump inhibitor treatment (at least 7 days).</p>
<p>Primary outcomes</p>	<p>Including saliva pepsin concentration, pH monitoring, GORD symptom questionnaire, Reflux Symptom Index, Voice Handicap Index, and Reflux Finding Score.</p>
<p>Statistical methods</p>	<p>Descriptive statistics are presented as mean±SEM for parametric data and median values with range where appropriate. The independent samples t test or Mann-Whitney U test was used to compare median values. The Wilcoxon signed-rank test was used to compare repeat questionnaire scores for patients. Correlation was performed using the Pearson test. The Fisher exact test was used for proportional differences. A p-value of <0.05 was considered significant.</p>
<p>Participants</p>	<p>Patients (21) with hoarseness and 'positive' laryngoscopy who had been diagnosed with laryngo-pharyngeal reflux on the basis of clinical evaluation (questionnaires) and laryngoscopy were included.</p> <p>Controls (n=10), adults with no symptoms of GORD or voice disorders.</p>

<p>Results^a</p>	<p>5 samples from each subject were analysed. In the patient group, there were 28 positive pepsin samples from 13 of the 21 patients (62%). In the control group, there were 6 positive samples from 4 of the 10 controls (40%). Only 1 of the 10 controls had ≥ 2 samples positive for pepsin compared with 9 of the 21 patients. The saliva samples taken from patients were more likely to be positive ($p=0.025$).</p> <p>Diagnostic accuracy of Peptest comparing the patients with the controls was not reported but it can be derived as the following based on the above data:</p> <ul style="list-style-type: none"> • in patients (cases and controls) with at least 1 positive pepsin sample — calculated based on number of patients: sensitivity 62%, specificity 60%, LR for positive test 1.55 LR for negative test 1.57 and false positive rate 40% • in patients (cases and controls) with at least 1 positive pepsin sample — calculated based on number of samples: sensitivity 27%, specificity 88%, LR for positive test 2.22, LR for negative test 1.20, and false positive rate 12%. <p>The above calculations presume that all the controls had been confirmed to be true negative by the same reference standard used for the patients.</p>
<p>Conclusions</p>	<p>The authors concluded that 'a subgroup of patients with hoarseness (10/21) had objective detection of the oesophago-pharyngeal reflux. We propose that these patients are more likely to benefit from further intense anti-reflux therapy. Detection of pepsin in the saliva may be a useful screening tool in these patients.'</p>
<p>Abbreviations: GORD, gastro-oesophageal reflux disease; LR, likelihood ratio; n, number of patients; SEM, standard error of the mean.</p> <p>^aOnly those outcomes that are relevant to this briefing were reported in this table.</p>	

Table 4 Summary of abstracts

Study component	Description
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Hayat et al. (2013)	
Objectives/ hypotheses	To measure pepsin in saliva with objective assessment of GORD by MII-pH in a cohort of asymptomatic patients and consecutive patients with clinically significant heartburn (according to the Montreal definition of GORD).
Tests	Index test: Peptest for pepsin in saliva (the cut-off value to determine pepsin positivity was 25 ng/ml). Reference test: MII-pH monitoring.
Participants	65 consecutive patients with clinically significant heartburn and 100 healthy people. Patients were divided into 3 phenotypes based on MII-pH results: <ul style="list-style-type: none"> • GORD, i.e. increased oesophageal acid exposure time (AET) (10.4 %±1.4) and SAP positive (n=26). • HO, i.e. normal AET and SAP positive (n=18). • FH, i.e. normal AET and SAP positive for acid/non-acid reflux (n=12).
Results	All healthy people selected had normal MII-pH testing. 36/100 healthy patients had at least 1 sample positive (20% had 1 sample positive, 12% had 2 samples positive and 4% had 3 samples positive). In pepsin positive samples, the median (25%>75%) pepsin concentration was 118 (64–181) ng/ml. In GORD patients, 21/26 had at least 1 sample positive (3 patients had 3 samples positive) and pepsin concentration was 152 (72–250) ng/ml. In HO patients 15/18 had at least 1 sample positive (4 had 3 samples positive) and pepsin conc. was 250 (74–250) ng/ml. In contrast, only 2/12 FH patients had at least 1 sample positive and pepsin concentration was 76 (67–85) ng/ml. Peptest had a sensitivity of 95%, specificity 89%, PPV 97% and NPV of 57% for identifying patients with heartburn related to reflux (GORD+HO).
de Bortoli et al. (2013)	
Objectives/ hypotheses	To evaluate the Peptest accuracy for the diagnosis of GORD in patients with reflux symptoms by means of MII-pH.

Tests	Index test: Peptest for pepsin in saliva/sputum (cut-off value for positive not stated) Reference test: MII-pH.
Participants	35 patients with GORD symptoms were studied. All patients with negative endoscopy underwent patho-physiological examinations. Patients were grouped on the basis of MII-pH results as follows: <ul style="list-style-type: none"> • true-GORD, i.e. increased AET/reflux number (n=16) • hypersensitive oesophagus (HO), i.e. normal AET/reflux number, positive SAP index) (n=12) • no-GORD patients, i.e. normal AET/reflux number, negative SAP index) (n=7).
Results	Peptest was positive in 93.7% of true-GORD, in 58.3% of HE, and negative in 100% of no-GORD patients. The accuracy of Peptest: sensitivity 100%; specificity 79%; positive predictive value 100%; negative predictive value 54%; diagnostic accuracy 83%.
de Bortoli et al. (2012)	
Objectives/ hypotheses	To evaluate the Peptest accuracy by means of MII-pH for the diagnosis of GORD in patients with reflux symptoms.
Tests	Index test: Peptest for pepsin in saliva/sputum (cut-off value for positive not stated). Reference test: oesophageal manometry and MII-pH.

Participants	<p>26 patients with GORD symptoms (all underwent upper endoscopy and, if negative, patho-physiological oesophageal examinations). Patients were grouped on the basis of MII-pH results as follows:</p> <ul style="list-style-type: none"> • true-GORD, i.e. increased AET/reflux number (n=11) • hypersensitive oesophagus HO, i.e. normal AET/reflux number and positive SAP index (n=7) • no-GORD patients, i.e. normal AET/reflux number and negative SAP (n=8).
Results	<p>Peptest was positive in 82% of true-GORD, in 57% of HO, and negative in 100% of no-GORD patients. It showed 72% sensitivity, 100% specificity, 100% positive predictive value, 62% negative predictive value, and 81% diagnostic accuracy.</p>
<p>Abbreviations: AET, acid exposure time; GORD, gastro-oesophageal reflux disease; HO, hypersensitive oesophagus; LR, likelihood ratio; MII-pH, impedance-pH; NPV, negative predictor value; n, number of patients; PPV, positive predictor value; RSI, reflux symptom index; SAP, symptom association probability index; SEM, standard error of the mean.</p>	

Recent and ongoing studies

One ongoing or in-development trial on the Peptest was identified in the preparation of this briefing:

- [NCT02183961](#): Comparison of 3 methods used in the diagnosis of extraesophageal reflux in children with chronic otitis media with effusion (enrolment: 24; study start date: June 2012; study completion date: May 2014).

Costs and resource consequences

If patients provide 3 Peptest samples at different times of day, as recommended by the manufacturer, the cost per test is £60 if the processing, testing and assessment is carried out by RD Biomed, or between £30 and £54 if processed and tested locally.

According to the National Schedule of Reference Costs for England, over 2011–12 there

were 164,679 diagnostic endoscopic procedures on the upper gastro-intestinal tract in patients aged 19 years and older, at a total cost of £71,829,933 with an average unit cost of £436.

The Peptest may provide a cost saving if a positive test result replaced the need for an endoscopic procedure. However, this will not be the case for all endoscopy referrals. The total potential cost savings would depend on the proportion of current endoscopy referrals that could be avoided where endoscopy was not indicated for other reasons, the proportion of those patients with acid reflux and the sensitivity of the test. A negative test result, either because the patient does not have GORD or the Peptest failed to diagnose it, would put the patient back on the pathway to current diagnoses using endoscopy or pH monitoring. In this case, the Peptest would be an additional care cost for these people.

Using Peptest could replace ambulatory pH monitoring where this test is carried out. According to NHS Tariff costs 2014–15 Disorders of the oesophagus with a length of stay of 1 day or less (FZ31F) has a unit cost of £392. It is unclear from the evidence how many such cases may be avoided by the use of Peptest.

Strengths and limitations of the evidence

In order to assess the usefulness of a new diagnostic test in clinical practice, evidence is needed to show:

- how well the test works compared to a reference diagnostic test that is known to work well in clinical practice
- the diagnostic yield of the test when it is introduced into a clinical pathway with other diagnostic tests
- the therapeutic yield associated with the test, that is, the benefits in terms of patient outcomes from the introduction of the test.

Current evidence on the Peptest only partially addresses the first of these questions.

In the Ocak et al. (2015) study, pH monitoring analysis and pepsin detection test analysis were double-blinded by separate researchers. However, the study was a very small cross-sectional study of 20 people with laryngopharyngeal reflux, and the recruitment method was unclear. Therefore, it is unclear whether the people in the study were representative of the population for whom the Peptest is intended, or whether the setting

was relevant to current NHS practice.

As the authors stated, pH monitoring is an imperfect reference standard. Furthermore only 1 sample was taken from each patient and this does not reflect how Peptest would be used in practice.

Hayat et al. (2015) was conducted in the UK and so the results may be more relevant to the NHS. The study had a relatively large sample size of 238 patients recruited consecutively, of whom 111 had symptoms of GORD and 100 did not. Three saliva samples were taken from each patient. The test accuracy was not included in the study report but has been calculated based on a subset of the patients in the study and their samples. Analysis was performed blinded to the patients' status, any reflux monitoring parameter and Reflux Disease Questionnaire scores. The test population consisted of patients with some primary symptoms of GORD. The publication did not state whether the included patients were people whose GORD symptoms had already failed to respond to proton pump inhibitors prior to their inclusion in the study. It was therefore unclear whether these patients matched the patients in whom Peptest might be used in clinical practice, because in practice it may be offered to people who have typical GORD symptoms and whose GORD had failed to respond to proton pump inhibitors and lifestyle changes.

The Hayat et al. (2014) study was also conducted in the UK, meaning the results may be more relevant to the NHS. It was a further small study including 31 patients. As with the Ocak et al. (2015) study, it was unclear how the patient sampling was done. It was also unclear whether the technicians were blinded to the status of samples, and to the order and timescale of testing.

The inclusion and exclusion criteria were somewhat different between the 3 studies, resulting in different populations and implications for interpretation of results. The various reference standards used in the studies, including MII-pH results and endoscopy examinations, are imperfect in classifying GORD.

A range of salivary pepsin concentrations were used as cut-off values for pepsin positivity in the samples across the studies, from greater than 16 ng/ml to more than 25 ng/ml. This makes it difficult to compare the results across the studies. There is no consensus in the published literature about standard and clinically relevant concentrations of pepsin in saliva. There was no comparable test for salivary pepsin, although laboratory tests were available and used primarily in a research context.

Between 1 and 5 tests were done per patient in different studies, and the calculated sensitivity and specificity are based on the number of patients tested. Pepsin positivity varied by time of day and proximity to eating. Furthermore, the 3 studies published as abstracts provided only very limited information in terms of study setting, methods, characteristics and results.

Overall, published evidence on the diagnostic accuracy of the Peptest for GORD and laryngopharyngeal reflux is of limited quantity and relevance. No published evidence on test characteristics for diagnosing GORD or laryngopharyngeal reflux from saliva or sputum in children was identified. No published evidence or useful information was identified to inform a clear role of the Peptest in a specific clinical pathway.

Relevance to NICE guidance programmes

The use of the Peptest is not currently planned into any NICE guidance programme.

NICE has issued the following guidance:

- [Dyspepsia and gastro-oesophageal reflux disease: Investigation and management of dyspepsia, symptoms suggestive of gastro-oesophageal reflux disease, or both](#). NICE guideline CG184 (2014). Date for review: September 2016.
- NICE guideline on [gastro-oesophageal reflux \(GOR\) in children](#). NICE guideline NG1 (2015)
- [Referral guidelines for suspected cancer](#). NICE guideline CG27 (June 2005, last modified April 2011).

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Search strategy and evidence selection

Search strategy

1. Databases were searched from inception to January 2015 including MEDLINE(R) In-Process & Other Non-Indexed Citations and MEDLINE(R) (via Ovid); Embase (via OVID); Cochrane Library; CAB Abstracts; Web of Science Science Citation Index. The keywords peptest, lateral flow test, saliva, sputum, pepsin, GERD, GORD, and reflux were used for the searches.
2. The internet was searched using the above keywords.
3. ClinicalTrials.gov, WHO ICTRP, and Current Controlled Trials were also searched for ongoing trials.
4. Information provided by the manufacturer was thoroughly checked for relevant studies. Information provided by the manufacturer in supporting this briefing was checked to identify any further information.
5. The manufacturer's website was thoroughly investigated.

Evidence selection

The references and citations from the above searches were sifted through to find any relevant material, using the inclusion criteria as follows:

Patients

Adults or children with suspected GORD. Potentially, the test could be used in secondary

care settings (to confirm whether symptoms or diagnoses may be related to GORD) or in primary care (to confirm symptomatic diagnosis of GORD).

Intervention

Peptest diagnostic test.

Comparator

When considering diagnostic test characteristics Peptest could be compared with:

- other point of care tests (if available)
- clinical diagnosis
- endoscopic diagnosis (this could be considered the reference standard for GORD diagnosis; however, NICE guidance lays out referral criteria, as a clinical diagnosis is often appropriate and confirmation of diagnosis by endoscopy is not always needed)
- other tests used in standard clinical practice to diagnose reflux
- diagnosis by treatment effectiveness of antacids and others.

Care pathways where Peptest could be introduced as an additional or optional test include:

- initial presentation of dyspeptic symptoms
- failure of initial antacid treatment
- before prescription of proton pump inhibitors
- to know whether to refer to endoscopy
- instead of referral to endoscopy (unlikely as endoscopy will also identify more serious pathologies including cancer and Barrett's oesophagus)
- to determine following initial investigations whether GORD is the cause of symptoms or conditions
- to determine whether GORD is the cause of non-dyspeptic symptoms or conditions.

Outcomes

Sensitivity, specificity, positive predictive values, negative predictive values, diagnostic yield and therapeutic yield.

Changes after publication

October 2015: Minor maintenance

About this briefing

Medtech innovation briefings summarise the published evidence and information available for individual medical technologies. The briefings provide information to aid local decision-making by clinicians, managers, and procurement professionals.

Medtech innovation briefings aim to present information and critically review the strengths and weaknesses of the relevant evidence, but contain no recommendations and **are not formal NICE guidance**.

Development of this briefing

This briefing was developed for NICE by [name of External Assessment Centre]. The [interim process and methods statement](#) sets out the process NICE uses to select topics, and how the briefings are developed, quality assured and approved for publication.

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