SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion and scientific discussion on procedures, which have been finalised before 1 October 2001. For scientific information on procedures after this date please refer to module 8B.

1. Introduction

Pylobactell is an orally administered *in vivo* diagnostic product, designed to investigate patients' status with regard to gastroduodenal *Helicobacter pylori* infection.

The active substance in this product is 100mg ^{13}C-urea, i.e. urea labelled with the non-radioactive stable isotope ^{13}C. It is presented in the form of a soluble tablet to be dissolved in water prior to oral administration. The diagnostic principle is based upon the urease activity of *Helicobacter pylori*. In the case of gastroduodenal *Helicobacter pylori* infection, the ^{13}C-urea is metabolised by urease and $^{13}CO_2$ is liberated in the exhaled air. Breath samples are collected and the $^{13}CO_2$ / $^{12}CO_2$ ratio is determined; it is this ratio that provides a quantitative indicator of *Helicobacter pylori* infection. Since other urease-producing bacteria are seldom found in the gastric flora, the detection of $^{13}CO_2$ in the breath above a certain limit is indicative of the presence of duodenal *Helicobacter pylori* infection.

Two clinical trials in a total of 366 patients have been described. In these trials a high diagnostic efficiency of the breath test following ingestion of ^{13}C-urea was shown, independent of use after or before *Helicobacter pylori* eradication therapy, and with due regard to the specified parameters of test meal, dosage and cut-off point of the assay. The clinical studies reported in the dossier used isotope ratio mass spectrometry (IRMS) to analyse breath samples, although any other objectively qualified method may be applied, provided it is suitably validated for use with this product by a competent laboratory.

None of the clinical studies performed with the product reported side effects due to ^{13}C-urea. In view of the fact that urea is intrinsically present in the body and only a small additional amount is to be administered in the form of this product, it is considered to be safe.

Although Pylobactell is a diagnostic test to detect *Helicobacter pylori* infection with a high specificity and sensitivity, differential diagnosis with invasive endoscopic methods might be indicated in order to examine the presence of any other complicating conditions, e.g. ulcer, autoimmune gastritis and malignancies. It should also be kept in mind that the performance of the test would be affected by treatments, which may interfere with *Helicobacter pylori* status or urease activity, e.g. antibiotics or proton pump inhibitors, and these restrictions are set out in the SPC.

2. Chemical, pharmaceutical and biological aspects

Pylobactell is submitted as multi-component test kit, for the *in vivo* diagnosis of gastroduodenal *Helicobacter pylori* infection.

Composition

The active substance is presented in the form of an uncoated, soluble, oral tablet containing 100 mg of ^{13}C-urea and small amounts of pharmaceutically well-accepted excipients. Other elements in the kit enable the efficient administration of the tablet and collection of breath samples to be sent for analysis.

Pharmaceutical Development and Product Manufacture

The aim of the development studies was to achieve a simple and rapidly dissolving formulation so that the urea is presented to the gastroduodenal flora in solution. A tablet dosage form was chosen, in which the active ingredient is directly compressed with the excipients. The tablet is to be dissolved in domestic drinking water immediately prior to administration, and this justifies the use of the standard term 'soluble tablet' in this case. The resulting solution is almost tasteless.

The active substance is hygroscopic and therefore precautions are taken to reduce humidity during the manufacturing process. In addition, this has determined the choice of container system and each tablet is individually packed in a heat-sealed PET/aluminium foil/LDPE laminated sachet for maximum protection from moisture, although urea is known to be chemically very stable in this regard and no significant degradation can be expected.

A simple direct-compression process carries out manufacture of the tablet, and these are transported to another site for assembly of the total test kit.

GMP Statement

See Section II.1 of this report.

Active Substance

^{13}C-urea is synthesised by a simple one-stage process, which has been known and published since the 1960's, using ^{13}CO, sulphur and ammonia. The ^{18}O content of ^{13}CO is controlled to a satisfactory level, as this may carry through to the active substance and product, and may in theory influence the result of the breath test.

The manufacturer of the active ingredient is different from the MA applicant, and therefore a DMF has been provided during the evaluation phase. Satisfactory high quality has been demonstrated in terms of batch analyses. It complies with the PhEur monograph for Urea with appropriate additional tests, in particular the isotopic purity with regard to the ^{13}C-label is specified as equal to or greater than 99%. Satisfactory stability has also been shown.

Finished Product Control

The tablet is tested for description, identification of urea by LC (& MS with regard to ^{13}C-urea), thickness, diameter, and average weight, uniformity of weight, hardness, moisture, disintegration time, urea assay (LC) and ammonia content, by validated methods. A test for microbial contamination according to Ph Eur is also included in the specifications.

Information on the solubility of the tablet under the conditions of the test (i.e. in tap water, with shaking) indicates that dissolution is complete in 2-3 minutes. This is considered to be so rapid that routine control in the specification is not necessary.

Stability of the Finished Product

Urea is a chemically stable substance, and ^{13}C is a stable non-radioactive isotope of carbon, so no problems were anticipated with the chemical and isotopic aspects of stability. Dissolution rate does not change significantly when the product is stored as defined in the SPC.

The stability studies presented in the dossier confirm this general view. Samples from earlier stability studies were reanalysed using a newly developed method for the determination of related degradation products e.g. biuret. The results support the proposed shelflife of 2 years, and the shelflife specification includes suitable limits for biuret and ammonia.

The company is committed to providing the stability results of the first three production scale batches on an ongoing basis, when available, for further confirmation.

3. Toxico-pharmacological aspects

In summary, approx. 1.1% of expired CO_2 is in the form of $^{13}CO_2$. The daily intake of ^{13}C amounts to 2.7 g. The daily production of urea in man is about 30 g. Normal plasma concentrations of urea are 100–500 mg/l (1,7-8,2 mmol/l). The total body urea pool can range from 700 to 2000 mg and is not likely to be influenced by the single oral intake of 100 mg of ^{13}C-urea. Calculations show that the endogenous ^{13}C-urea pool ranges from 7 to 22 mg in a 70 kg adult. Total daily intake of ^{13}C is estimated at approx. 3g and so a single oral intake of 100 mg of ^{13}C-urea is not likely to be significant in terms of safety risk.

The evidence presented points to the conclusion that there are no safety concerns arising from the ^{13}C-label, urea or ^{13}C-urea itself, at the doses and dosage frequency proposed in the SPC for this product.

4. Clinical aspects

The core of the documentation supporting the diagnostic properties of the Pylobactell test consists of a retrospective analysis of test results collected in two clinical studies of clarithromycin in combination with omeprazole for eradication of *H. pylori* in patients with duodenal ulcers.

Based on an analysis of test results from 950 visits by 366 patients, a high sensitivity and specificity are reported.

The performance of the test during different test meals was studied in a clinical trial in which six different alternative meals were compared to the standard test.

The study population was limited to patients with duodenal ulcers. No children or patients with atrophic gastritis or gastric resection are included.

Pharmacodynamics and Pharmacokinetics

The dossier on clinical pharmacology is based on a literature survey of the dynamics and kinetics of ^{13}C-urea relevant to the performance of the test procedure, based on the conversion of urea to CO_2 by *H.pylori* urease, according to the following simplified scheme:

$$CO(NH_2)_2 \quad + \quad H_2O \quad \rightarrow \quad CO_2 \quad + \quad 2NH_3$$

Possible problems, which could arise through the presence of other bacteria than Helicobacter pylori, are discussed. In the studies referred to, the urease activity of washed mucosa was measured. Mucosa of the stomach as well as from different parts of the bowel was used although activity was highest in the stomach. The influence of other sources of urease that occur elsewhere in the gut, for example in the contents of the jejunum, has been discussed.

Non-*Helicobacter* bacteria have usually a much lower activity of urease, if ever. Other *Helicobacter* species also possess some urease activity and could in theory give a false positive result if these organisms are present in the stomach. This is considered to be not a substantial problem since they may also cause a disease that responds to the same modes of treatment as *Helicobacter pylori* itself. Concerning the possible presence of *H. Heilmanii*, it is known that this organism causes gastritis, however of a milder grade than *H. Pylori*. As it has not been possible to culture *H. Heilmanii* in humans, it has not been possible to compare urease activity between the two organisms.

In order to maximise the performance, some test parameters should be carefully adhered to in order to increase the contrast between *Helicobacter pylori* positive and negative patients so that the discrimination between the two groups of patients also increases. This can be achieved by choosing the optimal

- dose of ^{13}C-urea,
- test meal (quality and quantity),
- threshold level of the increase of $^{13}CO_2/^{12}CO_2$-ratio in breath and
- time schedule for the actions
  - baseline breath sample
  - test meal,
  - ^{13}C-urea-drink and
  - second breath sample.

In order to avoid distortions caused by food-derived CO_2, patients should rest during the test period.

A significant increase in the ratio $^{13}CO_2/^{12}CO_2$ is measurable after 30 minutes and the second breath sample is therefore recommended 30 minutes after taking the baseline breath sample.

Analysis of CO_2 in breath:

The increase of $^{13}CO_2/^{12}CO_2$-ratio is called the isotopic excess or “excess”, also referred to as

$\delta\ ^{13}C$. Clinical studies referred to in the dossier have used isotope ratio mass spectrometry (IRMS) to

determine this excess, and summary analytical details are given in the SPC, although alternative suitably validated methods may also be used.

Dose

The basis for the dose recommendation (100 mg ^{13}C-urea) rests chiefly on published evidence that the overall excretion characteristics of $^{13}CO_2$ over the range 75-350 mg of ^{13}C-urea are similar.

Test meal

The introduction of a test meal aims at delaying gastric emptying, in order to prolong the contact time between *Helicobacter pylori* and urea. Literature reports substantiating the value of the test meal in general have been submitted, and a clinical trial has focussed on the effect of this variable on the outcome of the test.

Time interval

A significant increase in the ratio $^{13}CO_2/^{12}CO_2$ is measurable after 30 minutes and the second breath sample is therefore recommended 30 minutes after taking the baseline breath sample.

Cut-off value

The increase of $^{13}CO_2/^{12}CO_2$-ratio of 3.5 has been set as threshold between *Helicobacter pylori* positive and *Helicobacter pylori* negative subjects. After evaluating the two clinical trials, this threshold has been obtained retrospectively in order to optimise and balance both sensitivity and specificity.

Influence of food and exercise

Ingestion of ^{13}C-isotopes by daily food is known to have a significant influence on the $^{13}CO_2/^{12}CO_2$ in breath. This is why by examining the $^{13}CO_2$ increase in breath after the administration of ^{13}C-labelled urea the measuring of the breath's baseline value is necessary in order to register only the ^{13}C-excess in CO_2 produced by the metabolised ^{13}C-urea.

After the intake of food enriched with ^{13}C and exercise the $^{13}CO_2/^{12}$ CO_2-ratio increases. This is the result from the differences in ^{13}C-content of fat and carbohydrate. These differences are bigger in people with a diet that contains many carbohydrates derived from plants, (maize, cane sugar). Also, the production of CO_2 derived from food, especially that from carbohydrates increases with exercise. To avoid distorted values resulting from differences in ^{13}C-content in food, in fat and carbohydrate, the level of exercise before the first and the second sample should be similar.

In order to standardise these variables, the SPC advises performing the test under fasted conditions, after a standard meal, and in a sitting position for most of the test, i.e. at rest.

Efficacy

In general, the applicant's Expert Report correctly reflected clinical trial data.

The clinical data supporting this application fall into three parts:

- Data from two multicentre clinical trials
- Data from routine clinical use of the test in a single centre
- Test meal study

Data from two multicentre clinical trials

Primarily designed to evaluate clarithromycin in combination with omeprazole in the healing of duodenal ulceration and the eradication of *Helicobacter pylori*. To be included in the analysis, the patients had to have at least one visit with a UBT (urea breath test) as well as at least one biopsy-based test. 366 patients who altogether provided data from 950 visits fulfilled this requirement.

It is notable that the pharmaceutical form used was a powder. No clinical trials have been performed with the soluble tablet, which is the subject of this application, Pylobactell. However, bioequivalence between the two forms is accepted since both are administered to the patient in the form of a solution. Study protocols have been provided with reference to the statistical part of the breath test, and a full study report has been submitted.

In general, most patients had more than one visit. Only those visits were evaluated where the breath test was performed as well as gastric biopsy (CLO, histology and/or culture). Each patient served as his/her own control. Patients had to have an empty stomach before the breath test was carried out.

(1) GCP

Considering the study protocols and study report together with a certificate from the sponsor led to the overall conclusion that the two multicentre studies have been performed according to GCP.

(2) Patients' characteristics

In the clinical trials only *Helicobacter pylori* positive patients were included and followed up during and after their eradication therapy. Therefore no untreated *Helicobacter pylori* negative patients were tested there. It was accepted that this does not seem to decrease the trials' value. Considering the population studied, statements concerning the restriction of the test to patients over the age of 18 years and patients with partial gastrectomy are included in the SPC.

(a) Data from routine clinical use of the test in a single centre

Uncontrolled study. Data from 674 tests in 514 patients have been collected. The increase of $^{13}CO_2/^{12}CO_2$ excess, post urea, ranged from –0.5 to 96.1 with the majority of tests being either clearly negative or positive. The tests in which there were anomalies between test results or in which the increase of $^{13}CO_2/^{12}CO_2$-ratio was in the range 3.5 to 5 were listed in the Expert Report. These cases could be explained adequately, for example by interactions with omeprazole and antibiotics. These data merely demonstrate that patients in clinical practice have been investigated with a urea-based breath test, and have not played a major part in establishing the satisfactory efficacy and safety of this product.

(b) Test meal study

Paired data on 129 human volunteers to assess the test meal used to delay gastric emptying and possible effect of endoscopy prior to performing the breath test. Almost exclusively performed on *Helicobacter pylori* positive patients. To validate the use of different test meals to delay gastric emptying during the UBT procedure six alternative meals were compared with proprietary brands of the standard test meal - 50 ml Calogen and 50 ml Ensure (25 g long-chain triglycerides, 7 g carbohydrates, 275 kcal). One hundred and twenty-nine subjects were recruited from patients with a positive CLO-test at routine gastrointestinal endoscopy (n=71), from patients attending for routine UBT (n=51) and from the staff of the clinic where the study was performed (n=7). The test was performed on two consecutive days using the standard meal or one of the alternative meals in a randomised order. The breath samples were collected at 20, 30 and 40 minutes after the administration of ^{13}C-urea. Twenty-one patients were given Calogen and Ensure on both test days. The alternative test meals were (number of subjects):

- 100 ml full cream milk (n=13)
- 100 ml butterscotch flavour Calogen (n=17)
- 4 g citric acid made up to 100 ml with water (n=12)
- 200 ml pure orange juice (n=56)
- 200 ml pure orange juice with 2.4 g added citric acid (n=13)
- 100 ml pure orange juice with 100 ml water (n=6)

The main question to be answered was whether the use of different test meals will affect the breath test procedure and subsequently change the *H. pylori* test result of the patient.

It is evident that there is a close association between the magnitude of the $\delta^{13}C$ excess value and the difference between the test meals, i.e. the higher the absolute 30-minute value, the higher the difference at 30 minutes.

Taking all patients together (disregarding the actual test meal) it is demonstrated that the variability between the test meals is dependent on the level of the $\delta^{13}C$ excess with only minor differences close to the cut-off point, indicating a low risk for a change of the *H. pylori* status between different meals. Orange juice can be recommended as an alternative to the standard meal.

Endpoints and their clinical relevance:

i) Comparison with a 'gold standard'

All the clinical data originally provided by the applicant have been recalculated using biopsy-based techniques as a 'gold standard'.

The individual test results were compared to the *H. pylori* status at each visit. For each diagnostic test the number of positive, negative, false positive and false negative results were determined against *H. pylori* status, using three different methods. From this, the sensitivity and specificity values for each individual diagnostic test were calculated. The methods are detailed below:

a. If all biopsy-based tests carried out at a visit were positive, then the visit was classified Positive for the presence of *H. pylori*

b. If all biopsy-based tests carried out at a visit were negative, then the visit was classified Negative for the presence of *H. pylori*.

c. Any visit in which anomalies occurred between the biopsy-based tests used was evaluated by three different methods:

1. If two biopsy based tests were positive, then any negative response was described as False negative and the *H. pylori* status classified Positive

   If two biopsy-based tests were negative then any positive response was described as false positive and the *H. pylori* status classified as Negative

   If there were only two biopsy based tests available and one test indicated a positive response, then the negative response was classified as false negative and then the *H. pylori* status classified as Positive.

2. If only one biopsy based test indicated a positive response, then the negative responses were described as false negative and then the *H. pylori* status was classified as Positive

3. If two or more of the tests were positive, then any negative responses were described as false negative and the *H. pylori* status described as Positive (provided successive visits did not indicate the contrary).

   If only one test indicated a positive response, then following visits must have clearly shown a positive *H. pylori* status in order for that visit to be assessed as *H. pylori* positive. If the following visits clearly indicated a negative *H. pylori* status, then the visit was classified as *H. pylori* negative (provided no further treatment was administered).

The following table depicts the estimates of specificity and sensitivity for the three methods. For estimates based on at least 100 observations the lower one-sided 95% confidence limit is given within parenthesis.

	Specificity (%) (95% lower conf. limit)			**Sensitivity (%) (95% lower conf. limit)**		
	method 1	**method 2**	**method 3**	**method 1**	**method 2**	**method 3**
Prestudy visit	75.0	100.0	100.0	98.0	95.1	98.3
				(96.3)	(92.8)	(96.7)
4-6w follow-up	92.7	92.7	94.3	97.8	97.8	100.0
	(87.6)	(87.6)	(89.6)	(95.0)	(95.0)	(98.3)
6-m follow-up	91.3	91.3	94.3	94.6	94.6	100.0
	(85.2)	(85.2)	(89.0)	(90.0)	(90.0)	(97.6)
12-m follow-up	100.0	100.0	100.0	100.0	100.0	100.0

The results for the three methods are very similar, demonstrating some degree of robustness. All reliable specificity estimates (based on more than 100 observations) are between 91.3% and 94.3%, and all sensitivity estimates are above 95%. Thus, good diagnostic properties during different conditions have been demonstrated. The above sensitivity and specificity estimates are reflected in the SPC.

ii) The cutoff value, 3.5

A detailed substantiation of the cut-off value of the test did not emerge from the clinical trial data described above. Instead, a review of published references was undertaken to assess ^{13}C excess cut-off values and other parameters used by other investigators. In a retrospectively designed study, the results from seven multi-national studies were analysed (1229 tests). Additionally, 1815 and 935 tests from clinical trials and routine clinical data were added into the database. These studies had used similar protocols and the effect of using an excess ^{13}C cut-off value of 3.5 rather than 5.0 was investigated. Receiver Operating Characteristic (ROC) analysis of this total database indicated that a cut-off value of 3.5 excess provided the greatest specificity and sensitivity when employing a Calogen & Ensure test meal, 100 mg ^{13}C urea and 30 minutes post-urea breath sampling. On this basis, a cut-off point of 3.5 is considered an optimal balance, which leads to increased sensitivity without decreasing the specificity.

Safety
None of the studies reported in the dossier reported side effects due to ^{13}C urea.

In general, as neither the ^{13}C isotope nor urea has any pharmacological or toxicological effects, the test may be repeated in the same individual, within the limitations imposed in the SPC.

5. Conclusions

Satisfactory quality of Pylobactell has been demonstrated, allowing a 2 year shelf life when stored at room temperature, (below 25^{o}C).

In view of the fact that urea is intrinsically physiologically abundant and only a small additional amount is to be administered under the conditions of this test, it is considered that any additional risk is negligible. None of the clinical studies performed reported side effects due to ^{13}C-urea.

The Pylobactell ^{13}C-urea breath test procedure is a non-invasive method to detect gastroduodenal *H. pylori* infection. The clinical trials reported show a high efficacy of procedure with regard to variables such as test meal, dosage and cut off-point, independent of use after or before therapy of *H. pylori* infection. The average sensitivity and specificity for the procedure, compared to biopsy-based techniques was > 95% and > 90% respectively. The standardisation of the breath test as defined in the SPC makes this method suitable for general clinical use especially in patients with known gastritis or gastroduodenal ulcer disease.

The breath test reflects *H. pylori* status and can be used as a control after an eradication therapy. However, for the differential diagnosis before *H. pylori* eradication therapy, invasive endoscopic examination and histologic analysis of biopsy specimens are essential in order to examine the presence of any other complicating conditions (e.g. ulcer, autoimmune gastritis and malignancies).

The suppression/eradication of *H. pylori* may lead to false negative or positive results, therefore the test should be used after at least 4 weeks without systemic antibacterial therapy and 2 weeks after the last dose of acid antisecretory agents such as proton pump inhibitors.