Name of research institute or organization:

Pelikan Technologies GmbH & Co. KG

Title of project:

High altitude study with glucose biosensors

Project leader and team:

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Project description:

1. Background

PTG (Pelikan Technologies GmbH and Co. KG) develops glucose biosensors to measure the blood sugar of diabetic patients in capillary blood. Aim is to develop very small sensors that can work with a minimal blood volume (60 nl), preferably in combination with an integrated, electronic lancing technique. A very small blood volume is generated using pain reducing electronic lancing technique; the tiny blood drop is directly captured by the biosensor and the glucose concentration is measured. No milking of the finger or additional handling steps are required.
The integrated system will largely reduce the pain of the patient and increase the comfort when testing blood sugar. A more comfortable testing should increase the frequency of testing of patients and contribute to a better adjustment of the blood sugar level, thus increasing the health of the patient.

The PTG biosensor uses an amperometric detection principle, based on the enzyme GOD (glucose oxidase) in combination with a mediator. The mediator is used to make the reaction independent from the oxygen partial pressure of the sample. The natural reaction partner of GOD would be oxygen, regenerating the enzyme (fig.1, left). In the PTG sensor a mediator replaces the oxygen (fig.1, right). The regeneration of the mediator creates the current (nA) that is detected and used to calculate the glucose concentration of the sample.
If oxygen competes with the mediator, the sensor can show an oxygen sensitivity. The higher the oxygen partial pressure of the sample is, the lower the current reading would be. Hence at high altitude (and low oxygen partial pressure) too high current readings corresponding to too high glucose concentrations would be detected. This may result in a too high insulin dose provided to the diabetic person, eventually resulting in severe harm.

Before a product can be launched, preclinical and clinical trials must be conducted to verify the acceptable performance of the biosensors. During the preclinical trials it is to be verified that the biosensors show acceptable performance at the claimed altitude (for PTG: 3000 m). In the experiments at the high altitude research station Jungfraujoch the performance of the biosensors at high altitude was tested with blood and control solution to evaluate the system performance in advance of preclinical studies.

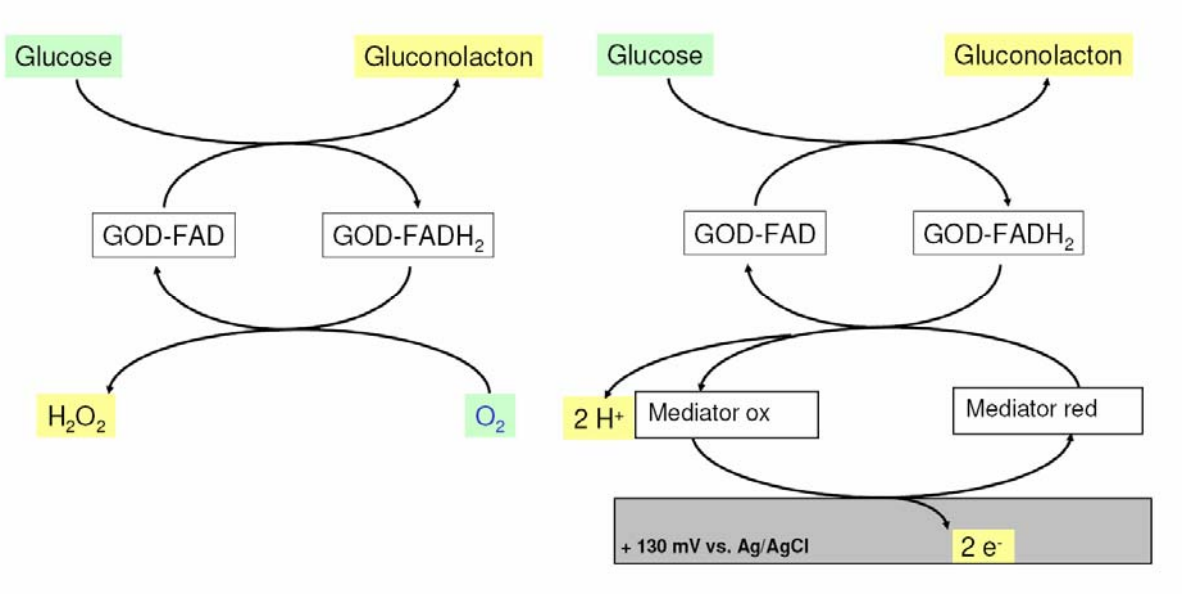

Figure 1: Amperometric glucose detection using glucose oxidase (GOD) as enzyme.
Left: Natural regeneration of GOD by oxygen. Right: Regeneration of GOD using a mediator.

2. Experimental

Aim of the experiments was to analyze if blood glucose readings of PTG sensors are accurate and precise at altitudes of >3000 m. Experiments with PTG control solution (AS#5), with venous blood after equilibration with oxygen in air and with capillary blood (small user study) have been performed. Experiments have been performed at low and high altitude and results at different altitudes were compared. Low altitude experiments were performed in the Pelikan Laboratories in Münster, high altitude experiments were performed at the high altitude research station Jungfraujoch.

Control solution experiment
Three different control solutions (60, 120 and 240 mg/dl) were each analyzed with 20 readnings on PTG sensors. At high altitude more than 95 % of readings must be within +/- 20 % bias of a seperatly determined low altitude average value. The low altitude average was calculated from 180 single readings per solution. The test with 20 readings per solution was performed three times at low altitude (to evaluate the best possible system performance) and four times at high altitude.

Experiment with venous blood
Venous blood of one volunteer was spiked to five different glucose levels (60, 120, 180, 250, 350 mg/dl), enriched with oxygen by agitation in air and analyzed with each 25 readings on PTG sensors and 10 readings on HemoCue (glucose reference device). The average bias between PTG sensors and HemoCue reference was calculated for each sample and must be below +/- 15 % at low and at high altitude. CV at low and high altitude must differ less than 5 %. The experiment was performed three times at low altitude and one time at high altitude.

Capillary user study
Five volunteers were tested in a user study set up. Each volunteer was tested twice on each 12 finger sites during an experiment (first test fasting and second test after sugar intake to increase the glucose span). From each of the 24 wounds per test person three readings were taken (in total 72 readings per volunteer per experiment). The study was performed with the same five volunteers for three times at low altitude and for two times at high altitude. The altitude bias (bias between studies at low and at high altitude) must be below +/- 15 %.

3. Summary of results

Control solution experiment
Only one experiment at high and one experiment at low altitude passed the acceptance criterion of > 95 % of readings within +/- 20 % bias. This is due to the fact that the CV with control solution is largely enhanced compared to blood. The results with control solution are therefore not reliable enough to draw serious conclusions on altitude effects. The AS#5 control solution needs to be redesigned to guarantee lower CV's on the PTG glucose biosensors.

Experiment with venous blood
All experiments at low and high altitude passed the acceptance criterion 1 (less than +/-15 % average bias to reference measurements with HemoCue for all analyzed samples). As well the second criterion was passed: The CV's at high and low altitude show less than 5 % difference. The PTG biosensors show no altitude effect according to the acceptance criterion for oxygen enriched venous blood.

Capillary user study
For each wound the average reference value (mean of three HemoCue readings) was compared to the three single measurements on PTG sensors from the same wound. The bias for each single reading on PTG sensors to the average reference value was calculated. The average bias for each volunteer and the whole study population was calculated. The altitude bias (difference of average bias at low and at high altitude) was analyzed per volunteer and for the whole population.
At high altitude all volunteers show some positive off set of the glucose readings compared to low altitude, indicating a mild oxygen effect. The average altitude bias is 6 % for the whole study population (table 1). The acceptance criterion (altitude bias for single user < +/- 15 %) has been passed for all volunteers. The mild oxygen effect of the PTG biosensor does not result in a critical altitude effect.

Table 1: Average bias to reference at low and at high altitude for each volunteer. Altitude bias (difference between bias at low and at high altitude) for all volunteers. Bias and altitude bias as average values over all volunteers.

Average Bias to reference [%]			
User	Low altitude	High altitude	**Altitude bias**
1	-8,8	1,2	**10,0**
2	-9,7	1,2	**10,8**
3	6,5	7,4	**0,9**
4	4,7	11,0	**6,3**
5	-2,3	-0,4	**1,9**
All Users			
MV (Bias) in %	-1,9	4,1	**6,0**

4. Conclusions

In the high altitude study at Jungfraujoch it could be shown that the PTG glucose biosensors show now relevant altitude effect. All observed altitude effects were well below the acceptance criteria of +/-15 % bias. Therefore the risk to get wrong readings at high altitude for diabetics using the sensor is acceptable. It is not expected that during preclinical trials the product should fail due to inacceptable altitude effects.

Key words:

Glucose, biosensor, high altitude, oxygen

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