Feature Article
BIOPEP Core Technologies and Know-how
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Biopep, which joined the HORIBA ABX Group in August 2003, specializes in cutting edge peptide-based technology used principally in hemostasis and having direct applications for the new Pentra 400. Biopep also possesses ultra-modern research facilities and brings to the Group its hi-tech reagent expertise and capacity to develop innovative diagnostic applications.

Introduction
A company specializing in hemostatic reagent research and development, BIOPEP develops, manufactures and markets peptide-based diagnostic products for medical research and analytical and industrial laboratories. In these markets, BIOPEP is recognized for its specific expertise in colorimetric tests using enzymatic methods. In August 2003, BIOPEP joined the HORIBA Group, becoming a 100% subsidiary of HORIBA ABX now working together to develop innovative applications.

Hemostasis, a Leading Discipline
Hemostasis is the science concerning coagulation mechanisms that control the cessation of bleeding from blood vessel lesions. Hemostatic anomalies often explain the causes of diseases such as hemorrhage or thrombosis (blocked blood vessels), which are the cause of cardiac infarction, pulmonary embolism, cerebral embolism or phlebitis. We should recall that cardiovascular diseases are the leading cause of death in industrialized countries.

In the field of hemostasis, BIOPEP intends to improve laboratory work: for biological laboratories, this means better test accuracy and stability, as well as kits which are very easy to use; for industrial or research laboratories, BIOPEP has R&D resources for developing new, specific, high-performance working tools.

The Thrombin/Prothrombin System
This system regulates blood fluidity. It involves several mechanisms, the principal one being to increase plasma viscosity through the formation and aggregation of fibrin which, in the event of dysfunction, leads to the formation of clots. Fibrin synthesis results from the cleavage of fibrinogen by thrombin, the key enzyme in coagulation. Thrombin results from the activation of prothrombin by Factor Xa, itself arising from a series of activations by other coagulation factors.

Figure 1 shows the coagulation cascade and the fields of action of BIOPEP.

Blood coagulation is regulated by a balance mechanism that on the one hand protects individuals against bleeding by forming blood clots, and on the other hand maintains plasma fluidity, thus preventing the blockage of blood vessels.
**BIOPEP**

**Peptide-based Expertise**

In a diagnostic context, peptide substrates are modified at the C-terminal end in such a way that the action of proteolytic enzymes releases a stained or fluorescent product (Figure 2).

Two types of derivatives manufactured by BIOPEP are mainly in use today: chromophore substrates and fluorescent substrates.

With respect to chromophore substrates, the enzyme’s hydrolytic activity triggers the release of p-nitroaniline (PNA), which is quantified by spectrophotometry at 405 nm. The same activity on an AMC peptide releases 7-amino-4-methylcoumarine, which is quantified by fluorescence.

However, another research objective is to develop electrogenic substrates, to provide new methods of detection in whole blood.

**BIOPEP Focusing on a 4-pronged Development Approach**

- Design and production of new diagnostic kits to analyze pathologies linked to hemostasis and thrombosis, in a fast-growing global market.
- Design and production of new peptides, much more efficient than those currently available, which will form the basis of high-performance diagnosis kits. For information, these peptides are "biological keys" which recognize perfectly the target biological molecules which are to be measured and therefore play an essential role in the quality of diagnostic assays to be performed or in drug development.
- Adaptation of BIOPEP reagents to a presentation or format which meets the workload of all laboratories, both large and small.
- Development of tools and technologies needed to develop direct tests on whole blood.
BIOPEP Products Today

- Range of diagnostic kits (CE certified)
  The Chromopep kits provide a range of reagents for determining the various parameters involved in hemostasis (heparin, protein C, antithrombin, plasminogen, etc.). Each of these methods provides improvements in terms of linearity, stability, accuracy and practical use (Table 1).

<table>
<thead>
<tr>
<th>Item</th>
<th>Competitors</th>
<th>BIOPEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>0 to 1 IU/mL</td>
<td>0 to 2 IU/mL</td>
</tr>
<tr>
<td>Stability</td>
<td>1 month</td>
<td>3 months</td>
</tr>
<tr>
<td>Accuracy</td>
<td>4%</td>
<td>2%</td>
</tr>
</tbody>
</table>

- Range of deep-frozen products (CE certified)
  Although all the major operators on the market offer freeze-dried products, BIOPEP offers its customers a really new product concept. This range of deep-frozen plasmas, ready to use in 5 minutes, eliminates the lyophilizing stages and their disadvantages, thereby avoiding any change in product quality as well as errors during reconstitution. Stored at -40 to -80°C, these plasmas have a shelf-life of 3 years and can be used up to 8 hours after defrosting, with a level of quality which is comparable to that of fresh plasma from healthy donors.

- Range of research products
  BIOPEP offers more than 100 references in proteins, enzymes, peptides and chromogenic substrates. Apart from this range of standard products, BIOPEP’s expertise and production equipment are used for customized synthesis of peptides, chromogenic substrates, proteins and enzymes for specific laboratory requirement, while strictly following the requirements of the European (E.P) and North American (U.S.P) Pharmacopoeias.

BIOPEP’s Industrial Equipment

BIOPEP currently works in 1,600 sq.m. of laboratory space and has developed an ultra-modern technical platform. Operations are organized around 3 main centers.

Chemical Laboratory

The chemical laboratory is equipped with all the material necessary for synthesizing peptides (fume cupboards, HPLC, lyophilizers, evaporators, etc.). Synthesizing a peptide requires a great many successive stages (coupling, evaporation, purification) to produce an end product. There are many synthesis techniques used to produce quantities from one mg to 10 grams. In the end, production of a peptide will have involved a dozen cycles over a period of about 20 days.

Biochemistry Laboratory

BIOPEP has recently created a biochemical laboratory in order to develop new diagnostic kits which will expand its range. This laboratory has the equipment needed to accurately identify and measure enzyme activity (spectrophotometry, HPLC, electrophoresis, etc.). These tools are intended to expand the principle of photometry measurement to other assays for which measurements are still made by determining coagulation times (chronometric tests), as well as continuing to develop innovative techniques that work directly on whole-blood and creating tests which meet specific “point of care” market requirements.
Production and Quality Control

A diagnostic kit contains several flasks holding enzymes and substrates or plasmas. All these constituents must be produced within the requirements of very strict quality and production norms, with strict testing at every stage of the production process. Today, each batch produced is used for several hundred kits, but production could be multiplied by 4 using the existing industrial resources. At the end of the line, BIOPEP can pack and ship its kits to different customers, with perfect traceability of batches and kits.

Conclusion

BIOPEP is therefore one of very few companies with a unique expertise currently only claimed by major diagnostics companies. Thanks to its highly competitive edge, BIOPEP uses all its assets to offer its customers high-quality, original products with high added value. Growth is guaranteed by the major synergy between BIOPEP and HORIBA ABX in particular:

- The Group's skills, technologies and financial resources as well as the size of its international distribution network.
- Their joint ability to develop technologies or methods directly on whole blood.
- HORIBA ABX's skills in developing new instruments to enable all laboratories to use automatic analyzers, to forge ahead even further in their field of diagnostics.