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TECHNICAL INFORMATION

Catalog Number: 102056
Intrinsic factor

CAS # : 9008-12-2

Definition: The product is a glucoprotein extracted from the pylorus part of the gastric mucous membrane. Its presence is considered necessary for the absorption of Vitamin B-12.

Materials: MP Intrinsic Factor is produced from the pylorus mucous membrane of the pig, of which only a small part is used. Only the part which, according to histological examinations has proved to have a high content of Intrinsic Factor, is used.

Production: No chemicals are used during production, apart from a solvent used for defatting. The whole manufacture takes place at temperatures below 0ºC. These measures avoid a modification of the active principle which is very labile in the macerated gastric mucous membrane.

Physical Description: Amorphous, yellowish-white powder

Activity: The activity is normally indicated in Coli units, the number of which are proportional to the binding power of the substance to Vitamin B-12. The standard quality of MP Intrinsic Factor contains at least 50 Coli units per mg (total binding).

Determination of the Activity: The number of total Coli units is not sufficient to indicate the real activity of the preparation. The following trials should also be taken into consideration:

1. Microbiological test, Coli units: Although there is usually reference to the total binding, one ought to distinguish between the thermostable binding and the thermolabile binding, this latter one being the expression of the activity.

MP applies a method elaborated by Professor Hoff-Jorgensen, Copenhagen. His laboratory controls every batch that is produced, determining the proportion between the total number of Coli units and the thermolabile Coli units. If the number of thermostable coli units is too big, the lot is rejected, even if the total binding is satisfactory. The following example indicates the correct proportions:

Total binding: 50 Coli units per mg
Thermostable binding: 20 Coli units per mg
30 effective Coli units per mg

2. Biological and clinical tests: The microbiological determination of strength indicates only very poorly the therapeutic activity. It is only by testing the preparation on patients, who are suffering from pernicious anaemia, that its value can be estimated. It is not possible to undertake such determinations as a routine control, but they are regularly undertaken by our supplier to re-examine the constant quality of MP Intrinsic Factor.

3. NF-units, Schilling test: A NF-unit is the oral and daily dose of a product, consisting of Intrinsic Factor and Vitamin B-12, which is given to people suffering from the pernicious anaemia of Addison, and it contains enough Intrinsic Factor to keep the clinical and haemotopoetic condition on a satisfactory level. The preparation should contain 10 mcg cyanocobalamin in the daily dose.

It has been possible to control the dosage by means of the Schilling test, as a standard preparation containing one oral unit in 50 mg has been used for comparison, and which has been placed at disposal by "The American Pharmaceutical Association". Such a standard preparation has been out of use since 1965, so you cannot with cause talk about an oral NF-unit. It is however possible, by means of the Schilling test, to find the daily dose of the preparation that is necessary in the treatment.

4. The Schilling test: It is based on measurements of the quantity of Vitamin B-12 that is resorbed in the intestine. These measurements take place by a determination of the quantity of B-12 that is secreted in the urine, before and after the Vitamin B-12 has been marked radioactively.

The patient who is undergoing the tests is given orally Vitamin B-12 without Intrinsic Factor, and after a certain time the above

determination is made. A little later the determination is remade, but this time after the patient has been given B-12 with Intrinsic Factor. To compare the different activities of the Intrinsic Factor preparation it is usual to make a series of determinations by giving the daily dose of a well-known preparation - it was formerly 50 mg of the official NF-preparation. The difference between the results should not exceed 20%. With reference to the standard preparation a daily dose of MP Intrinsic Factor corresponds to 35 mg, which has been proved by numerous Schilling tests as shown in the Following three examples:

	Percentage of B-12 secreted in the urine		
Patient	without Intrinsic Factor	with 50 mg NF-standard	with 35 mg MP I. F.
1	2,47	20,40	19,30
2	2,28	7,82	14,80
3	0.70	22,70	32,17

5. Clinical Control: The best way in which to estimate the value of an Intrinsic Factor preparation is to observe patients suffering from "classical" pernicious anaemia, and which are treated orally with this preparation. Our supplier is in continuous touch with several University Clinics where the activity of our standard preparation is constantly supervised. As a determination of our preparation may take several months it is not possible to examine every batch clinically, so we have to be contented with test-samples. It should be pointed out that in many clinics there are patients having been treated with our preparation for more than 10 years, and with very satisfactory results.

6. Formation of antibodies: After the use, over a certain period, of numerous Intrinsic Factor preparation on the market, some patients react by producing antibodies against Intrinsic Factor, and as a result of this the level of B-12 in the serum of the patient, which level has hitherto been satisfactory, will go down gradually to a point where it will be hardly sufficient, in some cases even pathological.

Researchers that have been made, and especially at the University Clinic of Vienna, have proved that the antibodies that the patients are producing during the treatment with out Intrinsic Factor have no influence whatsoever on the resorption of Vitamin B-12. From more than 100 patients that have been treated in the various clinics it has only been necessary to stop the treatment of 4 patients, and these persons were already suffering from other diseases, for this reason it cannot be confirmed that the observed effect is due to Intrinsic Factor.

Fields of Application: Intrinsic Factor is used in the treatment not only of classical pernicious anaemia, but also in the treatment of other macrocytic anaemias, where it replaces teh Intrinsic Factor which may be lacking in the patient's own gastric juice. In this way Intrinsic Factor is recommended against anaemias that appear 6 to 12 months after serious gastric operations, and against anaemias that are secondary effects of the treatment with certain medicines, e.g. against epilepsy. However, the dietetic use in multi-vitamin preparations is of the greatest importance. In older people there has been observed a degeneration of the gastric mucous membrane, whose secretion of Intrinsic Factor is insufficient to ensure the resorption of the necessary quantity of B-12 from their diet. In order to delay this development it is useful to prescribe a small quantity of Intrinsic Factor with B-12 and other vitamins to these patients.

Determination of Coli Units by E. Hoff-Jorgenson

Method: A culture of E. Coli bacteria, which as been isolated from the faeces of a patient with untreated pernicious anemia, is used. The culture is inoculated as a stick-culture on a substrate of the following composition:

Agar	2 g	
Extract of yeast (#103303)	1 g	
Peptone	1 g	Substrate I

Gluscose	1 g
Potassium phosphate sec.	1 g

The solution is made with water to a final volume of 100 ml (pH 7.3)

From the stick-culture is inoculated 50 ml sterile substrate in a conical flask. The composition of this substrate is:

NaCl	0,50 g	
K_2HPO_4	10,00 g	
$MgSO_4$	1,00 g	
$(NH_4)_2SO_4$	1,00 g	Substrate II
KCN	0,01 g	
Sodium Citrate, $3H_2O$	0,50 g	
Asparagine	4,00 g	

The solution is made with water to a final volume of 800 ml (pH 7.3)

The solution is autoclaved in portions of 40 ml. Before the use is added 10 ml 5% Glucose solution.

The inoculated substrate is placed at 30°C for 18-24 hours, and then the culture is separated by centrifuging in sterile tubes. The upper solution is discarded and sedimented cells are suspended in 20 ml of sterile Substrate II.

After determination of the turbidity in a part of the suspension the number of cells is calculated, and this calculation is based on a standard curve depicting the relation between turbidity and number of cells. Then the culture is diluted to a final concentration of 2×10^9 cells per ml.

In a sterile centrifuge tube the following is pipetted:

0,1 ml B-12 solution (normally containing 2 g B-12 per ml

0,1 - 0,9 ml of the solution whose amount of B-12 binding factor is to be determined

1 ml of the bacteria suspension with 2×10^9 cells per ml

Sterile water is added to a final volume of 2 ml

The tube is shaken for 2 hours at 30°C, and the solution is separated in centrifuge.

In the cell-free upper solution the amount of vitamin B-12 is determined by one of the known microbiological methods, after the B-12 has been liberated by heating for 10 minutes at 120°C in an autoclave.

The determination is repeated this time after heating the solution, whose amount of B-12 binding factor is to be determined, for 10 minutes at 80°C in a water bath before pipetting.

The difference between these two determinations is a measure for the heat labile B-12 binding effect.

Solubility: Slightly soluble in toluene; practically insoluble in water