ALLERGENS
for
diagnosis and treatment

Edited by
Prof. Bogdan Petrunov, M.D., D.Sc.
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Contemporary clinical and experimental allergology could hardly be envisaged nowadays without sufficient amounts of highly specific, effective, and safe allergens for diagnosis and treatment. Production of such preparations covers major environmental allergenic substances, including dust, bacteria, molds, pollen, food, etc., and their use is widespread in routine allergologic practice. Diagnostic applications in vivo (skin and inhalation tests) are based on specific immune reactions between allergens and allergenic antibodies fixed on skin or mucous membranes – IgE (reagins) or sensitized lymphoid cells. Such interactions result in development of local or generalized response, an equivalent of the allergic state. Use of allergen preparations for treatment purposes, the so-called specific hyposensitization (immune therapy), likewise relies upon immunologic underlying mechanisms, primarily formation in the patient’s body of protective “blocking” antibodies of the IgG class. These interfere with binding of environmental allergens entering the body to effector antibodies (reagins) and, along with other beneficial phenomena resulting from the treatment – such as enhancement of histaminopexy activity, decrease of the allergen – provoked histamine release by basophils and mastocytes, stimulation of T suppressor lymphocytes, and thus drop in IgE antibody levels, – lead to substantial improvement of the patient’s condition.
ALLERGENS FOR DIAGNOSIS AND TREATMENT

Allergen preparations provide opportunities to apply modern diagnostic and therapeutic techniques in allergic diseases. These diseases are assuming increased importance in human pathology, and the practitioner encounters almost daily one or another form of allergy: bronchial asthma, asthmatic bronchitis, hay fever, allergic rhinitis, urticaria, etc. It is thus necessary for him to be well aware of existing types of allergen preparations, their diagnostic indications and contraindications, their routes of administration, and their capabilities.

For diagnostic and therapeutic purposes, the Laboratory of Allergy, National Center of Infectious and Parasitic Diseases (NCIPD) produces seven basic types of allergens: house dust and dandruff, pollen, bacterial, food, fungal, occupational, and insect. Our allergens are designed for skin and provocation (inhalation, nasal, etc.) tests and for specific hyposensitization. The same allergens may successfully be used for a variety of in vitro immunologic studies. To this end, they may be delivered to the consumer in the desired concentration and form (liquid of freeze-dried).

Besides the allergen preparations included in our production list, the Laboratory of Allergy in NCIPD could produce also others, upon request of the customers, with the raw materials provided by them.

STANDARDIZATION OF ALLERGEN PREPARATIONS

Our allergen preparations for diagnosis and treatment are double standardized:

1. On the basis of weight/volume of the raw material and the extract solution, specific for each group of allergens.

2. Biological standardization – on the basis of the skin allergometry of each kind of allergen in comparison with a histamine standard – histamine hydrochloride in a Coca I solution + 0,4 % phenol + 0,02 % human serum albumin, in a concentration of 1 mg/ml for the pollen, mite and insect allergens, used for prick-test diagnosis, and 0,1 mg/ml for all other allergens, used for intracutaneous testing.

The activity of the allergens is expressed in BIOLOGICAL UNITS (BU). An allergen, which causes skin reaction similar in size to the one produced by the adequate histamine standard, possesses a biological activity equal to 1000 BU.

3. The pollen allergens are standardized (besides on the basis of weight/volume in the beginning of the extraction process) in protein nitrogen units (PNU) also. One PNU is equal to 0,00001 g protein nitrogen.

ALLERGENS FOR DIAGNOSIS

Diagnostic allergens are liquid extracts from basic sensitizing substances of various origin: domestic, bacterial, mold, pollen, alimentary, industrial, etc. They are intended for diagnostic purposes and administered in allergy skin tests. Production technology employs recent advances in allergology to isolate and purify allergizing constituents. Their qualities – specificity, safety, and
sensitivity – ensure that positive skin responses are obtained only in subjects who are sensitized to the respective substances from which the allergens have been prepared. They are very active and, in allergic people, cause appearance of marked, clear-cut and reproducible skin reactions. No irritating or toxic substances are contained, and no effect is produced in nonallergic individuals. A 5 ml diagnostic allergen vial suffices for 50-60 intradermal skin tests or about 100 prick tests.

Therapeutic allergens are designed to be used in specific hyposensitization treatment of allergic patients. They permit application of this most contemporary etiopathogenetic treatment method that in above 70% of cases leads to excellent or very good clinical results. A treatment set consists of six 5 ml vial with concentrations ranging from 0.1 to 1000 BU of a given allergen (for pollen allergens – a set of eight 5 ml vials with concentrations ranging from 1 PNU to 10 000 PNU). Administration is subcutaneous, with concentrations and amounts progressively rising over a 3-year period, as illustrated by the model schedule attached.

For maintaining cure, the set consists of three vials each containing 5 ml of the highest allergen concentration attained (commonly 1000 BU or 2500 – 5000 PNU).

The allergoids (chemically modified allergens) are the newest category allergens for specific immunotherapy (hyposensitization). As a result from chemical processing, they have lost or decreased significantly their allergization activity (with other words their ability to sensitize the human body with formation of specific antibodies from class IgE) and preserved their ability to stimulate the formation of specific “blocking” antibodies from class IgG. These qualities of theirs allow a significant improvement of the clinical results from specific hyposensitization to be obtained. This is due to the possibility a much higher dose of a given allergen to be applied in the treatment’s course with no risk of body’s allergization or appearance of adverse side reactions. Besides that a considerable simplification and shortening of the treatment’s course is achieved thanks to a decreased number of injections and increased interval between them.

The allergoids are particularly suitable for treating young children and patients with strongly manifested hypersensitivity to a certain allergen, which hinders latter’s application in the needed dose and concentration till a desired clinical effect is achieved. The allergoids for treatment are presented in 6-7 vials (5 ml) with concentration resp. of 1 PNU and 10 000 PNU from a given allergen. They are applied subcutaneously, gradually increasing the concentration and quantity, for a 3-year term according to the enclosed scheme.

For maintain treatment a set of 3 vials (5 ml) is produced with allergoid of the highest reached concentration during the treatment (usually 10 000 PNU).
Allergen preparations are fit for use during one year; they should be stored in a refrigerator at +4° to +8° C and protected from direct sunshine.

**DIAGNOSIS OF ALLERGIC DISEASES**

Essential to successful treatment of allergic diseases in accurate etiologic diagnosis – proper identification of responsible allergens. To achieve this, it will be necessary, subsequent to detailed allergologic history and comprehensive clinical examination, to perform a number of investigations with allergen preparations. Among the variety of test types available, including inhalatory, conjunctival, nasal, in vitro immunologic, and cutaneous, the latter have assumed particular importance and find extensive application in everyday clinical allergologic practice.

Allergy skin tests (AST) are simple to perform, require no special equipment, and are feasible at any health establishment; response scoring is rapid, and most importantly, they are highly specific and safe for the patient; hence, they provide an excellent diagnostic tool and are widely used throughout the world.

Allergy skin tests are to be undertaken only following complete history and careful clinical examination, and provided the patient is not in an acute stage of the disease (with asthmatic fits, for instance) as testing at such a time may aggravate his condition. It should be noted that to be valid, ASTs should be done at least three days after discontinuation of any glucocorticoid or antihistamine drugs since, otherwise, subdued skin response may cause false negatives.

**PRICK-TEST PROCEDURE**

On the volar surface of the forearm, after cleaning of the skin with ethyl alcohol, a drop of allergen is placed through which 2-3 pricks of the epidermal skin layer are performed using a syringe needle. The distance between two skin tests should be at least 3 - 3.5 cm, to avoid the mixing of the skin reactions produced. The patient’s arm should remain in a horizontal position until the reading of the result, thus avoiding spilling of the drops and mixing of allergens, which could lead to false skin reactions.

The reading of the Prick-test reactions is performed after 15-20 min. The size of the papule and erythema are outlined by a ballpen and read. The longest diameter of the papule (Ap) and erythema (Ae) are measured as well as the perpendiculars of these diameters (Bp and Be). The sum of those perpendicular diameters of the papule and erythema, divided by two gives the mean diameter of the papule (Dp) and erythema (De) which is used for expression of the results of this skin test.

\[
D_p = \frac{A_p + B_p}{2} \quad D_e = \frac{A_e + B_e}{2}
\]

The result of the skin allergic test represents a fraction with a **numerator** which is the mean diameter of the papule (Dp) in mm, and a **denominator** – the mean diameter of the erythema...
(De) in mm – Dp/De. Each reaction with a mean diameter of the papule and erythema above 3/3 mm is considered as positive. The reading of the skin reactions is performed according to the table (p. 20, Annex 1).

The intradermal allergic tests are performed using tuberculin or 1 cc syringe with gauge No. 19 or 20 tuberculin needles. The introducing of each allergen should be performed with different sterile syringes and needles not only because of the strong requirements for aseptic work, but also to escape the mixing of different allergens and the appearing of false skin reactions.

After cleaning with ethyl alcohol the volar surface of the forearm, the allergen is injected intracutaneously in a dose of 0,05 ml to form a wheal. It is advisable to avoid bleeding during the injection, which will jeopardize the exact reading of the skin reaction. The quantity of the allergen injected intracutaneously in 3-12 years old children should be 0,03 ml. The distance between two skin tests should be not less than 3-3,5 cm, in order to avoid the mixing of the skin reactions obtained. The zones situated 4 cm under the elbow joint and 4 cm above the wrist should be avoided during the performance of the skin tests.

The intracutaneous allergic tests are read after 15-20 min and after 24th hour. The size of papule (infiltrate) and erythema of the skin reactions obtained are measured after being outlined with ball-pen. The way of reading is absolutely analogous to those used in Prick-test. The final result is expressed as a fraction with a numerator the mean diameter of papule or infiltrate (Dp) in mm, and a denominator the mean diameter of erythema (De) in mm – Dp/De.

Each skin reaction with mean diameter of the papule (infiltrate) and erythema above 7/7 mm is considered as positive. The reading of the skin reactions obtained is performed according to the table (p. 20, Annex 1).

All allergens except the pollen, mite and insect (used by Prick-test) are applied intracutaneously. It should be borne in mind that in exceptional cases when there are data for a very high sensitivity of the patient or in children with allergic diseases all other diagnostic allergens could be applied by Prick-test.

Once the etiologic diagnosis has been secured, the question arises of how to treat the allergic patient. Present-day views on treatment consider three approaches:

1. Removing the patient from the allergizing environment or, inversely, removing to the largest extent possible the allergen from the patient’s environment;
2. Undertaking nonspecific hyposensitization to readjust general reactivity;
3. Undertaking specific hyposensitization (immunotherapy) with the allergen of interest to which the patient is hypersensitive, as indicated by his history and AST, and other in vivo and in vitro
methods. The first two approaches are not readily accomplished and may well be impracticable with some allergens and some patients, whereas specific hyposensitization is feasible with almost any allergen, the exception being alimentary allergens where results have been unsatisfactory. As regards house dust and dandruff, pollen, bacterial, fungal, occupational and insect allergen preparations, specific hyposensitization yields very good results. Provided the practitioner is well aware of indications and contraindications, procedures, and capabilities, results should be satisfying both for him and the patient. From reports in the literature throughout the world and our own solid experience with this method in management of inhalation allergy (bronchial asthma, asthmatic bronchitis, hay fever, and allergic rhinitis), 32 % of treated patients give excellent results, with complete relief from fits; 47 % achieve substantial reduction in intensity and frequency of fits; 10 % continue to have fits, with some decrease in intensity or frequency; and in no more than 11 % does the cure produce no effect. Clinical results are even better in the case of children, who are mostly monoallergic rather than polyallergic (lost specificity stage). As no other method of allergic disease treatment is capable of ensuring better results, specific hyposensitization is obviously of great practical value.

Patients may undergo specific hyposensitization concurrently with any other treatment for allergic disease. Performance within the framework of daily clinical practice is possible at any health establishment having a physician trained to apply the method, leaning on guidelines and with consideration given to the particulars of each patient. The individual approach to the patient is a basic principle never to be underestimated and resting upon his general conditions, his sensitivity, and his tolerance. Hyposensitizing therapy includes a basic cure and a maintaining cure.

**Basic cure.** - Injections are given strictly subcutaneously 2-3 times a week on a definite schedule, sequentially in the order of vials starting with the vial containing the lowest concentration (commonly 0,1 BU). With the last vial with the highest concentration (commonly, 1000 BU), treatment proceeds as follows: 0,4 ml of this concentration is given twice a week until the allergen is exhausted. Upon completion of the basic cure, the patient attends for a control examination and receives instructions as to his further treatment.

**Maintaining cure.** - This is carried out with the highest allergen concentration attained (commonly 1000 BU) as follows: 0,4 ml twice a week over a period of 2-4 months; 0,4 ml once a week over 10-14 months. Should the attending physician consider clinical response to be very good, he may expand the time interval between two consecutive injections to 10-14 days. It should be stressed that to obtain a steady therapeutic effect manifested in substantial relief from complaints, specific hyposensitization (basic cure plus maintaining cure) is to be con-
sistently carried out for at least three years.
In case where two responsible allergens have been identified, these allergens are administered simultaneously, with one injected into the right forearm and the other into the left forearm; separate syringes and needles are thereby to be used, instead of mixing allergens and administering them in one injection.
Should other illness (influenza, pneumonia, angina, renal infections and other diseases) intervene during the treatment period, hyposensitization is temporarily discontinued until the patient recovers. The cure then proceeds further by repeating, firstly, the last two allergen injections given prior to development of the intercurrent illness. If the discontinuation is more prolonged then the treatment could start again using an allergen concentration lower than that which has been reached before the illness.

Specific hyposensitization using pollen allergens is done on an annual basis, beginning with November-December (preseasonally) and following a definite schedule.

Basic cure.-- Injections are strictly subcutaneous, 2-3 times a week, consecutively by vial order, starting with the lowest-allergen-concentration vial (commonly, 1 PNU). Upon administrating the last dose of the highest concentration (5000 PNU), the cure continues as follows:
- to the end of May, 0.4 ml of the maximum allergen concentration attained once weekly
- June and July, 0.4 ml once in 10 days;
- August and September, 0.4 ml once in 14 days;
- October, November, December, 0.4 ml once in 20 days.
In November the patient attends for his control examination and prescription of subsequent treatment, which continues three years regardless of the clinical effect. This long period is absolutely necessary if steady therapeutic results are to be achieved.
Maintaining treatment of patients who have undergone the hyposensitization cure described above begins in January and includes the following:

<table>
<thead>
<tr>
<th>500 PNU</th>
<th>1000 PNU</th>
<th>2500 PNU</th>
<th>5000 PNU</th>
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<tbody>
<tr>
<td>0,1 ml</td>
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<td>0,6 ml</td>
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Injections are given twice a week until the highest allergen concentration is attained (commonly, 5000 PNU); after that the cure follows the calendar schedule indicated earlier.

Contraindications for undergoing specific hyposensitization: exacerbation of the basic allergic disease; intercurrent nonallergic disease - inflammatory or infectious affections, cardiac or
pulmonary failure, diabetes, damage to kidney or liver parenchyma, active tuberculosis, pregnancy.

Complications. — In dealing with allergen preparations, complications may develop, however rarely, in an oversensitized subject. They can be local, with appearance of erythema, edema, and pain at the site of injection; or they can be systemic, in which case provocation may occur of an asthmatic spell, tachycardia, itching over the whole body, disseminated urticaria, even collapse.

If such complications do occur, the physician is faced with the serious problem of how to go on with the specific hyposensitization cure. There are a number of alternatives for decreasing the amounts of allergen administered and in this manner overcoming adverse effects:

1. The time interval between two consecutive injections is prolonged by 3—4 or more days, depending on severity of untoward effects and tolerance of the patient.
2. The amounts of allergens administered per injection are reduced: in the above schedule, for instance, from 0.4 ml to 0.2 ml or 0.1 ml at the same concentration.
3. The same dose with the same concentration is repeated until patient tolerance improves.
4. In the case of more serious systemic reactions, one may go back not only to a lower dose but also to a lower concentration; for instance, from 1000 BU to 500 or 100 BU.
5. There are cases, particularly in specific hyposensitivity cures involving pollen allergens, where administration of 1-2 tablets of an antihistamine drug 30 min prior to the injection next in turn proves a very good means for overcoming either local or systemic reactions.

By making use of one of these schemes, or a combination of them, the attending physician will be able to proceed without risk with the specific hyposensitization cure. Particularly severe cases of local or systemic complications seriously affecting the general condition of the patient are treated, similarly to anaphylactic shock, with subcutaneous or even intramuscular 0.5—1.0 ml adrenalin injections, either with or without 0.5 mg atropine. Instead of adrenalin, 0.5—1.0 ml asthmo-physin may be given, slowly, by intravenous injection. Glucocorticosteroids and antihistamines must also be at hand for parenteral administration. To be used freely are all cardiac and peripheral stimulators such as strophantin, caffeine, corazole, etc., as well as desensitizing drugs, calcium, vitamin C, etc.

In carrying out allergy skin tests or specific hyposensitization, particular attention is to be given to the cleaning of syringes and needles, if no disposable syringes are available. Glass has namely the property to avidly absorb allergens. Normal washing being unable to remove the latter completely, syringes and needles that have been used with an allergen are to remain im-
mersed in water during 1 or 2 hours, then be washed 3 – 4 times in 1 % nitric acid, and finally abundantly rinsed in water. Such treatment is mandatory if mixing of allergens, and hence compromising of results, is to be avoided.

**Description:** The preparation represents a clear, colourless or yellow-brownish liquid of pH 6,8 – 7,2. It is a 10 % Coca I solution extract of the initial raw material (house dust, mites, dander, hair or human dandruff), containing 0,4 % phenol plus 0,02 % human serum albumin, standardized in biological units by allergometric titration in sensitized persons and by using histamine standards.

**Application:** It is applied for diagnosis – skin allergic or inhalation tests, and treatment – specific hyposensitization of the allergic patients.

**Dosage:** For diagnosis – 0,05 ml of the allergen is used in concentration of 1000 BU, intracutaneously. The mite allergen is used in Coca 50 % glycerol solution by Prick-test. The reaction is read on the 15-20th min and 24th hour by estimating the diameter of the papule and erythema in mm. The treatment is carried out according to a scheme with gradually increasing doses: from 0,1 ml to 0,8 ml and concentrations from 0,1 BU to 1000 BU.

**Expiration date:** One year after preparing the allergen.

**Storage:** In a dark place, at a temperature of +4°C to +8°C.

**Packing:** For diagnosis – vials containing 5 ml allergen of 1000 BU concentration (red labels). For hyposensitization – box, containing 6 vials of 5 ml allergen with concentrations of 0,1, 1, 10, 100, 500 and 1000 BU (green tables).

**Description:** The preparation represents a clear, colourless liquid (light yellowish when in concentration of 5000 PNU) of pH 7,8 – 8,2. It is a 7,5 % extract of different pollen species, containing 0,4 % phenol plus 0,02 % human serum albumin. It is chemically standardized in PNU and biologically, by allergometric titration in sensitized persons, by using histamine standard.

**Application:** It is applied for diagnosis – skin allergic or inhalation tests, and treatment – specific hyposensitization of allergic patients.

**Dosage:** For diagnosis – the allergen is used in 1000 PNU concentration by Prick-test on the volar side of the antebrachium. The reaction is read on the 15–20th min by measuring the diameter of the papule and erythema in mm. The treatment is carried out after a scheme with gradually increasing doses – from 0,1 ml to 0,8 ml and concentrations – from 1 PNU to 5000 PNU, subcutaneously, 2–3 times weekly.

**Expiration date:** One year after preparation of the allergen.

**Storage:** In a dark place, at a temperature of +4°C to +8°C.

**Packing:** For diagnosis – vials, containing 5 ml allergen of 1000 PNU concentration (red labels).
GROUP B “A”.

**GROUP POLLEN ALLERGOID**

*Description:* The preparation is a clear, colourless liquid (yellowish when in concentration of 10,000 PNU) with pH 7.8-8.2. This is a 1% extract of lyophilized group pollen allergen composed of the six most broadly distributed and significant for allergology in our country pollen types: D.glomerata, Festuca sp. Lolium perenne, Secale cereale, Phleum pratense, Arrhenaterum elatius. It is obtained by two-stage modification with formalin and containing 0.4% phenol + 0.02% human serum albumin chemically standardized in PNU biologically by dermal allergometry in sensitized patients utilizing histamine standard.

*Application:* It is applied only for treatment – specific hyposensitization of allergic patients to pollen.

*Dosage:* Treatment according to the enclosed scheme, gradually increasing the dose: from 0.1 ml to 0.8 ml, and concentration: from 1 PNU to 10,000 PNU, twice a week.

*Expiry date:* Keeps quality for a year after its date of production.

*Storage:* To be stored away from light and at temperatures from +4°C to +8°C.

*Package:* A box containing 6-7 vials (5 ml) with the allergoid in concentration: 1 PNU, 10 PNU, 100 PNU, 1000 PNU, 2500 PNU, 5000 PNU, 10,000 PNU (green label).

**GROUP C. FOOD ALLERGENS**

*Description:* The preparation represents a clear liquid of varying colour (depending on the initial product) in fixed concentrations, pH 6.8-7.2. It is a Coca I or Coca II solution extract of various foods, containing 0.4% phenol as preservative; biologically standardized in W/V and by allergometric titration in sensitized persons, by using histamine standard.

*Application:* It is applied for diagnosis – skin allergic or provocation tests.

*Dosage:* For diagnosis – 0.05 ml of a fixed allergen concentration is applied intracutaneously. The reaction is read on the 15-20th minute and 24th hour by measuring the diameter of the papule and erythema in mm.

*Expiry date:* One year after preparing the allergen.

*Storage:* In a dark place, at a temperature of +4°C to +8°C.

*Packaging:* For diagnosis – vials of 5 ml allergen of fixed concentration (red labels).

**GROUP D. BACTERIAL ALLERGENS**

*Description:* The preparation represents a clear, colourless liquid of pH 6.8-7.2. It is a complete Coca I solution bacterial extract from certain bacterial species, killed with formal (autolysate and bacterial body), containing 0.4% phenol plus 0.02% human serum albumin, standardized W/V and in number of bacterial bodies in ml, and biologically by allergometric titration in sensitized persons, by using histamine standard.
**Application:** It is applied for diagnosis—skin allergic and inhalation tests, and treatment—specific hyposensitization of allergic patients.

**Dosage:** For diagnosis—the allergen is used in a concentration of 1000 BU and 100,000,000 bacterial bodies per ml, applied intracutaneously in a dose of 0.05 ml. The reaction is read on the 15-20th min and 24th hour by measuring the diameter of the papule (infiltrate) and erythema in mm. The treatment is carried out according to a scheme with gradually increasing concentrations from 0.1 BU and 10,000 bacterial bodies per ml to 1000 BU and 100,000,000 bacterial bodies per ml, and doses ranging from 0.1 to 0.8 ml subcutaneously, 2-3 times weekly.

**Expiry date:** One year after preparing the allergen.

**Storage:** In a dark place, at a temperature of +4°C to +8°C.

**Packing:** For diagnosis—vials containing 5 ml allergen of 1000 BU concentration and 100,000,000 bacterial bodies per ml (red labels).

For hyposensitization—box containing 6 vials of 5 ml with concentrations from 0.1 BU and 10,000 bacterial bodies per ml up to 1000 BU and 100,000,000 bacterial bodies per ml (green labels).

**Description:** The preparation represents a clear, colourless or yellowish solution of pH 6,8-7,2. It is the extract obtained after the cultivation of the respective fungal species in synthetic liquid nutrient medium. It is standardized W/V and biologically by allergometric titration in sensitized persons, by using histamine standard.

**Application:** It is applied for diagnosis—skin allergic and inhalation tests, and treatment—specific hyposensitization of allergic patients.

**Dosage:** For diagnosis—0.05 ml of the allergen is used in a 1000 BU concentration, applied intracutaneously. The reaction is read on the 15-20th min and 24th hour by measuring the diameter of the papule (infiltrate) and erythema in mm. The treatment is carried out after a scheme with gradually increasing doses: from 0.1 ml to 0.8 ml and concentrations—from 0.1 BU to 1000 BU subcutaneously, 2-3 times weekly.

**Expiry date:** One year after preparing the allergen.

**Storage:** In a dark place, at a temperature of +4°C to +8°C.

**Packing:** For diagnosis—vials containing 5 ml allergen in a concentration of 1000 BU (red labels).

For hyposensitization—box containing 6 vials of 5 ml with concentrations of 0.1 BU, 1 BU, 10 BU, 100 BU, 500 BU and 1000 BU (green labels).

**Description:** The preparation represents a clear, colourless liquid of pH 6,8-7,2. It is a 10% Coca I solution extract of various industrial products, containing 0.4% phenol plus 0.02% human serum albumin. It is standardized in W/V and biologically by allergometric titration in sensitized persons by using histamine standard.
**GROUP G. INSECT ALLERGENS**

**Description:** The preparation represents a clear, colourless liquid of pH 6.8-7.2. It is a 0.1% Coca I solution extract, containing 0.4% phenol plus 0.02% human serum albumin. It is standardized in W/V and in biological units, by allergometric titration in sensitized persons, by using histamine standard. The honey bee allergen is prepared by the isolated and purified high molecular fraction from honey bee venom, consisting 85% of hyaluronidase and phospholipase A.

**Application:** It is applied for diagnosis - skin allergic tests and inhalation tests, and treatment - specific hyposensitization of allergic patients.

**Dosage:** For diagnosis - the allergen is used in a concentration of 1000 BU in 50% glycerol Coca solution and applied by Prick-test. The reaction is read on the 15-20th min by measuring the diameter of the papule and erythema in mm.

The treatment is carried out after a scheme of gradually increasing doses - from 0.1 to 0.8 ml and concentrations - from 0.1 BU to 1000 BU subcutaneously, 2-3 times weekly.

**Expiry date:** One year after preparing the allergen.

**Storage:** In a dark place, at a temperature of +4°C to +8°C.

**Packing:** For diagnosis - vials containing 5 ml allergen of 1000 BU concentration (red labels).

For hyposensitization - box containing 6 vials of 5 ml with concentrations of 0.1 BU, 1 BU, 10 BU, 50 BU, 500 BU, 1000 BU (green labels).

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**CONTROL SOLUTIONS**

During performance of skin allergic tests it is mandatory also to perform the following controls:

1. **Negative controls** (for absence of skin allergic reaction) - they are performed by using Coca I solution, containing 0.4% phenol plus 0.02% human serum albumin (in intracutaneous tests) and
50% glycerol Coca I solution plus 0,4% phenol plus 0,02% human serum albumin (in Prick-tests).

2. Positive controls (for presence of normal skin reactivity to histamine) – they are performed with histamine hydrochloride 0,1 mg/ml in a Coca I solution containing 0,4% phenol plus 0,02% human serum albumin (in intracutaneous tests) and with histamine hydrochloride 1 mg/ml in 50% glycerol Coca I solution, containing 0,4% phenol plus 0,02% human serum albumin (in Prick-tests).

The allergens for specific hyposensitization are delivered by the NCIPD Laboratory of Allergy upon submission by the patient of a document where data are entered relating to allergy skin tests, precise diagnosis, age and address, as well as payment for the preparation.

Each hospital, public health unit or medical doctor, which wants to obtain allergens for diagnosis should make an application to the NCIPD Laboratory of Allergy, indicating the type of allergen, number of vials, the bank account or the way of payment.

On questions regarding allergens quality, manner of storing and using, indications and contraindications, contact: Laboratory of Allergy NCIPD, 26 Yanko Sakazov Blvd., Sofia 1504, telephone 43-47-260.

For information on export of Bulgarian allergen preparations, please contact:

Medi Dental® Ltd., Georgi Sofijsky Str.
1431 Sofia, Bulgaria;
Tel. 00359-2-51 75 66; Fax 00359-2-52 15 06;
Tlx 24590 MEDENT

or

National Center of Infectious and Parasitic Diseases,
Laboratory of Allergy,
26 Yanko Sakazov Blvd.,
Sofia 1504, Bulgaria,
Tel. 00359-2-43-47-260; Fax 00359-3-44 22 60
A scheme for recording the skin allergic tests with inhalation, pollen, food, bacterial, fungal, occupational and insect allergens after 15-20 minutes and 24-48 hours

<table>
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<tr>
<th>Reactions</th>
<th>Intracutaneous test</th>
<th>Prick test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>after 15-20 min.</td>
<td>after 24-48 h.</td>
</tr>
<tr>
<td>Negative (-)</td>
<td>papule up to 7 mm</td>
<td>infiltrate up to 7 mm</td>
</tr>
<tr>
<td>Slight positive (+)</td>
<td>papule 7-10 mm and</td>
<td>infiltrate 7-10 mm</td>
</tr>
<tr>
<td></td>
<td>erythema 7-12 mm</td>
<td></td>
</tr>
<tr>
<td>Moderate positive (++)</td>
<td>papule 10-12 mm and</td>
<td>infiltrate 10-12 mm</td>
</tr>
<tr>
<td></td>
<td>erythema 10-12 mm</td>
<td></td>
</tr>
<tr>
<td>Strong positive (+++)</td>
<td>papule 12-15 mm and</td>
<td>infiltrate 12-15 mm</td>
</tr>
<tr>
<td></td>
<td>erythema 12-15 mm</td>
<td></td>
</tr>
<tr>
<td>Very strong positive (++++)</td>
<td>Every reaction above 15 mm</td>
<td>Every infiltrate above 15 mm</td>
</tr>
</tbody>
</table>