GREER DIA-KIT®

DIAGNOSTIC AID FOR HYPERSENSITIVITY PNEUMONITIS

A Gel Diffusion Kit for Demonstrating Precipitating Antibodies

For in Vitro Diagnostic Use
Store at 2°C to 8°C
Item # K10

A GEL DIFFUSION KIT FOR QUALITATIVE DEMONSTRATION OF PRECIPITINS AGAINST THE FOLLOWING ANTIGENS:

Thermoactinomyces vulgaris #1
Micropolyspora faeni
Aspergillus fumigatus #1
Aspergillus fumigatus #6
Aureobasidium pullulans
Pigeon Serum

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SUMMARY AND EXPLANATION OF TEST

The precipitin test dates back to 1897 with the double-diffusion plate test coming into use in 1948. As early as 1962, Pepys et al. demonstrated the presence of precipitins to moldy hay in the serum of Farmer's Lung patients. This finding served to link hypersensitivity lung disease with an inhaled antigen and the presence of precipitins against the antigen in the affected patient's serum. By examining a variety of organic dusts, investigators have isolated antigens such as thermophilic actinomyces, fungi and animal protein as causative agents for hypersensitivity pneumonitis.[1] Limitations of the test are discussed in a later paragraph.

PRINCIPLE OF THE TEST

The micro-Ouchterlony method of gel double-diffusion is used to demonstrate precipitating antibodies. The Dia-Kit® utilizes a gel plate with two patterns, each composed of a central well surrounded by six smaller wells. The central well is filled with the patient's serum and the peripheral wells with the various antigens. The antigens and the serum diffuse radially into the gel toward each other. If precipitating antibodies (usually IgG) to a given antigen are present, an antigen-antibody reaction occurs at the point of optimum concentration and a visible band(s) or arc is formed between the two wells.[2] The gel may be washed and stained to eliminate smudges and hazy precipitates.

CONTENTS OF DIA-KIT®—REAGENTS AND MATERIALS

1. Four gel immunodiffusion plates with two patterns per plate to allow for different antigen-antibody ratios. The agarose gel contains sodium chloride, citric acid, sodium phosphate and 0.1% sodium azide as a preservative. Each plate is enclosed in a sealed plastic bag and can be stored at 2°C to 8°C for six months.

2. A vial containing capillary tubes suitable for filling antigen wells.
3. Six antigens in screw cap vials.
   The following antigens were either reconstituted or diluted with saline containing 0.1% sodium azide as a preservative and Tris as a buffer. Store at 2°C to 8°C. Do not use if antigens show evidence of microbial contamination.
   a. ***Thermoactinomyces vulgaris #1*** (broth antigen) — 0.25 ml. Prepared from broth filtrate according to Edward's double-dialysis method.[3] The lyophilized extract was reconstituted at 20 mg/ml.
   b. ***Micropolyspora faeni*** (broth antigen) — 0.25 ml. Prepared from broth filtrate as described above.
   c. ***Aspergillus fumigatus #1*** (broth antigen) — 0.25 ml. Prepared from broth filtrate, lyophilized and reconstituted at 20 mg/ml.
   d. ***Aspergillus fumigatus #6*** (cellular antigen) — 0.25 ml. The mold was homogenized, lyophilized and extracted at 20 mg/ml. The extract was lyophilized and then reconstituted at 20 mg/ml.
   e. ***Aureobasidium pullulans*** (cellular antigen) — 0.25 ml. Extracted, lyophilized and reconstituted at 20 mg/ml.
   f. **Pigeon Serum**—0.25 ml per vial at 1:10 v/v.
4. Rectangular plastic film to be used during staining procedure.

CAUTION: Antigens and gel contain 0.1% sodium azide, which is highly toxic. Sodium azide reacts with acid to form a toxic gas and with metals in plumbing to form a volatile explosive. Avoid flushing used material down the drain.

SPECIMEN PREPARATION

1. Collect the blood sample by venipuncture and allow to clot at room temperature.
2. Separate the serum from the clot as soon as possible by centrifuging.
3. Store the serum at 2°C to 8°C if the test is not to be performed immediately, and store at -10°C to -20°C if the test is to be delayed more than 24 hours. Avoid repeated freezing and thawing of the specimen.

PROCEDURE

1. Materials Provided in Dia-Kit®:
   Gel plates, capillary tubes, 6 antigens, plastic film.
2. Additional Materials Required:
   Tuberculin syringe with 25-gauge needle.
3. Materials Required for Optional Procedures:
   a. Staining
      Spatula, forceps, Whatman filter paper #41, blotter, hair dryer, staining dish, distilled water, 0.15 M NaCI, methanol-acetic acid 0.2% Coomassie Blue.

b. Controls

Positive controls for Dia-Kit® antigens. See enclosed order blank.

4. Directions for Performance of Test:
   a. Open the small plastic box containing the gel on a flat surface. It is important that the plate remain on a level surface at all times.
   b. Fill the antigen wells (small peripheral wells) using the thin capillary pipettes provided. Fill the capillary pipette to about 2/3 full. Hold the pipette at a 45° angle. Touch the well with the lower tip of the pipette and then raise vertically to increase the flow of material into the well. Fill the well until no meniscus can be seen. Do not overfill because this may leave a ring of precipitate.
   c. Fill the serum well (large central well) using a large capillary pipette or a tuberculin syringe fitted with a 25-gauge needle.
   d. Close the box and carefully insert into the plastic bag. Reseal the bag and incubate the test plate at room temperature for 24-72 hours. Keep plates level during incubation.
   e. Observe daily for precipitin lines. Read the unstained plate using a viewer with a dark field background or hold at an angle to a fluorescent light.
5. Optional Washing, Drying and Staining Procedure:

   Since the Dia-Kit® gel does not adhere to the plastic box during washing and drying procedures, the following method is recommended.[4] It may be completed in approximately three hours.
   a. Use a spatula to loosen and transfer the gel to the hydrophilic side of the rectangular plastic film. A drop of water will spread rather than bead on the hydrophilic surface.
   b. Place the film on a flat surface. Fill the wells with distilled water and cover the gel surface with Whatman filter paper #41. Eliminate any air bubbles that may form between the gel surface and the filter paper. Then place a thick absorbent blotter (or fine textured laboratory towel) and a heavy book on the gel.
   c. Press for 10 minutes.
   d. Wash the pressed gel in 0.15 M NaCI for 15 minutes (with agitation). Wash with fresh saline for an additional 15 minutes.
   e. Wash for 15 minutes in distilled water.
   f. Press for 10 minutes as described above.
   g. Dry with a hair dryer (warm air-approximately 15 minutes) or in a drying oven at 60°C.
   h. Stain for 4-5 minutes with Coomassie Blue — 0.2% in methanol-acetic acid solution.
   i. De-stain with methanol-acetic acid solution (methyl alcohol — 450 ml., glacial acetic acid — 100 ml. and distilled water to 1 L). De-staining may require 2 to 3 changes of solution.

j. Dry with a hair dryer.

6. Controls:

   Although potency tests are performed on each antigen prior to release and at intervals during the dating period, it is recommended that positive controls be used to verify potency of antigens used in the procedure, and a negative control (normal human serum) is recommended for evaluating positive results. The gel plate was designed for the central well of both patterns to be filled with the same patient's serum. This procedure requires that additional plates be used for controls. However, the pattern with the smaller central well may be used to incorporate controls into the plate used for the patient's serum. If a negative control is desired, fill the smaller central well with normal human serum and the peripheral wells with the same antigens diffused against the patient's serum. If a positive control is desired for a given antigen, fill the smaller central well with the positive control serum and place the corresponding antigen in a peripheral well.

NOTE: Positive controls for Pigeon Serum and *M. faeni* antigens may be placed in a peripheral well adjacent to the corresponding antigen.

LIMITATIONS OF PROCEDURE

Precipitating antibodies against the offending antigen can be demonstrated in most cases of hypersensitivity pneumonitis. A positive test can provide the physician with a diagnosis when supported by historical and clinical evidence. A positive test does not necessarily indicate hypersensitivity pneumonitis since asymptomatic individuals may develop precipitins without any features of the disease. Nor does the absence of precipitins eliminate the diagnosis since precipitins are reduced or absent when the disease is not in an acute stage. Thus a positive test for precipitating antibodies is quite helpful diagnostically but should be interpreted in conjunction with clinical findings.[5] Furthermore, with the use of more sensitive gel diffusion techniques (e.g., counterimmunoelectrophoresis), serum precipitins are being detected against many ubiquitous organic dust, fungal and animal protein antigens in some of the "normal" population, perhaps merely reflecting continuous exposure of the population at large to such antigens.[6] The clinician must also consider the many limitations of gel diffusion techniques such as establishment of proper antigen-antibody concentration ratios, the need for protein stain and awareness of several types of false positive precipitin arcs in interpretation of results.[7-13]

If negative results are obtained with the antigens provided in the kit, further determinations should be made with other antigens.[14] See enclosure for other available antigens and positive controls.

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