MICROGEN BIOPRODUCTS

20

REF FSK4 AVIAN IMMUNODIFFUSION SYSTEM

CE

IVD

GB

INTENDED USE

The Microgen Bioproducts Avian Immunodiffusion System is a double gel diffusion assay intended for the detection of precipitating antibodies in human serum elicited in response to the inhalation of avian allergens. The diseases associated with the presence of these antibodies have been known variously as pigeon (or budgerigar or bird) handler's (or fancier's or breeder's) lung (or disease) depending on the source of exposure as well as the nomenclature adopted by the investigators. Turkeys, chickens and pheasants can also produce a similar condition and the term hypersensitivity lung disease to pigeons (or other avians) has been used. All these diseases form part of a group of diseases termed extrinsic allergic alveolitis.
The kit is intended for professional laboratory use only.

PRINCIPLE OF THE TEST

The precipitating antibodies detected by the kit are generated as part of the immune response to exposure to antigens derived from pigeons, budgerigars and poultry. These antibodies are predominantly IgG class, although other classes may have a precipitating function.
The test relies on the principle of double gel diffusion (Ouchterlony). When soluble antigens and homologous antibodies are placed in adjacent wells cut into suitable diffusion media such as agar or agarose, they diffuse towards one another and produce visible precipitation lines along the interface of optimal relative concentrations. A multivalent positive control antiserum containing suitable titres of the relevant antibodies can be tested in wells adjacent to the antigens being reacted with the patient's serum. The development of precipitin lines in control tests is used to confirm reagent and plate performance. With experience, the control can be used as a marker to indicate completion of diffusion. By using avian serum and faecal extracts, it is believed that the maximum number of all positive sera will be identified. Consequently, the diagnosis of hypersensitivity reactions should be made considerably easier.

KIT PRESENTATION

CONT			
CONTROL	+	FSK4/a	Positive Control: Sheep multivalent antiserum: 4.0mL
PIGEON SERUM		FSK4/b	Pigeon Serum: (prediluted) 0.5mL
BUDGIE SERUM		FSK4/c	Budgerigar Serum: (prediluted) 0.5mL
POULTRY SERUM		FSK4/d	Poultry Serum: (prediluted) 0.5mL
PIGEON FEX		FSK4/e	Pigeon Faecal Extract: (purified - 4mg/mL) 0.5mL
BUDGIE FEX		FSK4/f	Budgerigar Faecal Extract: (purified - 4mg/mL) 0.5mL

All reagents contain 0.099% sodium azide as a preservative.

Instructions for Use
Record Cards (20)

Additional Requirements:

- Double diffusion plates (Packs of 20 ready-to-use plates are available from Microgen Bioproducts - Product Code FSK/DDP)

WARNINGS AND PRECAUTIONS

Safety:

1. The reagents supplied in this kit are for *in vitro* diagnostic use only
2. Sodium azide, which is used as a preservative in the kit reagents can react with lead or copper plumbing to form potentially explosive metal azides. Dispose by flushing with a large volume of water to prevent azide build-up.
3. Appropriate precautions should be taken when handling or disposing of potentially pathogenic samples. Decontamination of infectious material can be achieved with sodium hypochlorite at a final concentration of 3% for 30 minutes. Liquid waste containing acid must be neutralised before treatment.
4. The antigen preparations have been inactivated during the manufacturing process. However, they should be handled as though potentially infectious.

Procedural:

1. The kit should be used according to these instructions.
2. During use, the reagents should be kept at ambient temperature for the minimum time possible.
3. Do not dilute any of the kit reagents
4. Do not intermix reagents from different batches of kits.
5. Do not freeze any of the kit reagents

STORAGE AND SHELF LIFE

The Avian Immunodiffusion System should be stored firmly stoppered at 2-8°C when not in use. Do not freeze. Any slight turbidity in reagents, particularly controls, will not normally interfere with satisfactory performance. The kit should not be used after the expiry date printed on the carton label.

SPECIMENS

5-10mL of venous blood should be collected and allowed to clot in a glass tube without anti-coagulant. Remove the serum and store at 2-8°C if the test is to be performed within 4-5 days. If longer storage is necessary, freeze the serum at -20°C to -70°C. Repeated freezing and thawing causes loss of precipitating antibody. Gradual decline in antibody titre is also to be expected if sera are stored at -20°C for longer than 6 months.

DOUBLE GEL DIFFUSION PLATES (Product Code FSK/DDP)

Each plastic envelope holds a plastic box that divides into two compartments separated by a foam layer. In each compartment, there is a glass slide coated with agar in which wells have been pre-cut. After use (see Test Procedure below), the stained, dried plates are suitable for filing as permanent records.

Chemical composition
Plates are prepared with 1.5% purified agar (Difco) in McIlvaine's citric acid phosphate buffer, pH 7.0 containing 0.099% sodium azide.

Dimensions
The slide is ca. 51x76mm and the agar gel layer is nominally 2mm thick. The large patient's serum well is 12.5mm in diameter with a nominal capacity of 200µL. The outermost antiserum control wells are 8mm diameter and will hold 100µL. The small 4mm antigen wells have a capacity of 25µL.

Filling the wells
Many micro-pipetting devices of both fixed and adjustable volume are commercially available and are recommended for loading the wells according to the volumes specified above. A fresh pipette or tip is essential for each serum specimen or antigenic extract. The large serum well size compared to the antigen well is to increase the amount of antibody available for reaction and is optimal for detection of weak precipitin reactions using unconcentrated patient's serum.

TEST PROCEDURE

Immunodiffusion

1. Bring the control sera and antigen extracts to room temperature. Gently mix any patient's serum that has been frozen.
2. Fill in a test record card with patient's data, number, wells to be used, contents, etc.
3. Remove the plastic box from the polythene envelope. Unhinge the box by gently twisting to form the two compartments. Remove the polythene foam layer if both compartments are needed, otherwise store the unused compartment (with foam) in the resealable envelope provided.
4. Paint the specimen number on the agar layer using indelible stain (e.g. 0.5% Alcian Blue) applied with a fine brush. The dye must be one that will not diffuse.
5. Fill the smallest wells with antigenic extracts. Both concentrations of the extract must be tested separately. The patient's serum is placed in the large well and appropriate control sera in the outer wells. Sufficient positive control is provided to fill 40 wells.
6. Place each compartment in a moisture chamber on a level surface and incubate at 35-37°C for 3 days or room temperature for 5 days. Plates should be examined daily for signs of precipitin lines. N.B. Resealable plastic envelopes are quite convenient, if moistened, for use as moisture chambers.

Washing and staining slides

7. Cut around the glass slide in each compartment with a scalpel blade or knife and raise up the glass agar plate. Place the slide in a dish or large beaker of saline (1% sodium chloride + 0.099% sodium azide). Wash the plate for at least 24 hours and preferably 48 hours changing the saline wash as frequently as is practicable.
8. Finally wash for 2-3 hours in distilled water.
9. Remove the slide, cover with filter paper and air dry at 37°C to reduce the gel to a thin film. Drying may be accelerated by careful use of a hair dryer. If necessary, moisten the filter paper to remove it. Re-dry the slide briefly afterwards.
10. Stain the slide in a dish of 0.5% Coomassie Blue B.L. (or equivalent) in methanol/acetic acid/water (5:1:4v/v) for 10 minutes.
11. Destain the slide in several changes of the same solvent for 15 minutes. Air-dry, record the results and file the slide.

N.B. Some users have found that a final 15 minute rinse of the slide in a 5-10% glycerol-saline solution enhances long-term preservation.

Reading and recording results

The unstained plates are best examined by viewing against a black background using transmitted light. Look for precipitin reaction lines in the area between the antigen wells and the patient's serum well. The positive control can be used as a marker for precipitin line development. The appearance of lines can be provisionally noted on the record card when diffusion appears complete but maximum sensitivity is obtained after full washing and staining.

INTERPRETATION

Precipitin lines should be visible between the positive control wells and the antigen wells. The presence of precipitin lines between the patient's sample and one or more antigen wells is indicative of a positive result.
No test should be reported as negative until the slide has been inspected following staining and destaining as faint precipitin lines may not be visible until this procedure has been completed. When the serum contains a large amount of antibody, the precipitin line will be close to the antigen well. Where there is very little antibody, the precipitin reaction is close to the patient's serum well. When more than one antibody is present, a series of precipitin lines may be visible; these can be used as a semi-quantitative guide to titre.
The precipitin titre of a positive serum may be determined by performing the double diffusion test with serial doubling dilutions of the sample.

Clinical significance

Precipitins against pigeon extracts: Precipitating antibodies against pigeon extracts are an aid to the diagnosis of pigeon fancier's lung. Their presence indicates exposure and an immunological response to inhaled avian antigens. The incidence of precipitins in symptomatic cases of the disease has been reported variously as 67-100% (3,4). However asymptomatic pigeon breeders may also have precipitins. In one survey, 40% of asymptomatic breeders had precipitins to a crude pigeon faecal extract and 20% to pigeon serum (5). The reaction alone therefore is not diagnostic and other clinical criteria need to be considered.
The relationship between antigens in pigeon serum and droppings has been studied and a non-specific reacting component in pigeon droppings has been identified. The Microgen Bioproducts pigeon faecal extract has been purified to eliminate non-antigenic material and is used at a dilution that does not produce non-specific reactions with normal human serum.

Precipitins against budgerigar extracts: Precipitating antibodies against budgerigar extracts are an aid to diagnosis of budgerigar fancier's lung. It has been reported that precipitins are detectable in 84% of cases of the disease whereas less than 5% of budgerigar handlers with other respiratory diseases exhibit precipitating antibodies (6). Tests with crude budgerigar droppings may produce reactions with sera of non-exposed individuals. The Microgen Bioproducts budgerigar faecal extract has been purified so that these non-specific reactions do not occur.

Precipitins against poultry extracts: Less information is available on poultry workers. Two cases of hypersensitivity lung disease to chickens had precipitins to chicken serum. However, 27% of a group of poultry farmers also had precipitins without apparent disease (7). A study in the turkey breeding industry has shown that 14% of workers with symptoms of lung disease exhibited precipitating antibodies compared to 5% of asymptomatic workers (8). In poultry handlers, the diagnosis of disease is mainly based on other criteria.

See Bibliography below for further information on correlation of precipitin line formation with avian antigen exposure.

LIMITATIONS OF USE

1. Results of a single test procedure should not be relied upon for diagnostic purposes but should be interpreted by the clinician in the context of all available clinical and laboratory information. Detection of precipitating antibodies alone does not constitute a diagnosis of active disease caused by avian antigens.
2. The presence of low levels of precipitins may simply indicate past or present exposure to avian antigens with no incidence of disease.
3. The absence of precipitins does not preclude the presence of disease caused by exposure to avian antigens
4. It has been shown that approximately 33% of patients with coeliac disease have precipitins in their sera to an antigen common to most avian sera and also present in hen egg yolk. The antigen is not associated with bird handler's lung and is not present in faecal extracts (9). Using double diffusion and low concentrations of avian sera as in the Microgen Bioproducts System, the coeliac antibody-antigen reaction is eliminated.

PERFORMANCE CHARACTERISTICS

There is no reference test procedure for precipitating antibodies to avian antigens with which to compare and evaluate FSK4.
750 serum samples have been tested with FSK4 and an enzyme immunoassay for IgG to avian antigens but correlation between the two sets of data is limited due to the following factors:

- Precipitating antibodies reacting in FSK4 may include classes other than IgG that will not be detected by the EIA.
- Some IgG antibodies detected by EIA may be directed against antigens not involved in the precipitating reaction. E.g. some low molecular weight antigens do not form precipitin bands.
- Not all IgG antibodies are precipitating.
- The EIA may not detect IgG to some antigens if they do not readily adsorb on to the surface of the microtitration plate.
- The EIA is generally more sensitive and will detect low levels of IgG. However, these levels may not be clinically significant. Higher levels of precipitating antibodies correlate with the presence of active disease.
- Diagnostically important levels of precipitating antibodies are not always detected by EIA. Similarly, relatively high levels of IgG in some sera may not be detected by FSK4.

The results of the comparative study (shown below) should therefore be reviewed and interpreted in the light of these factors. There are a number of discrepant results which may not have clinical significance. This emphasises that diagnosis of disease can only be made after considering the results of a range of tests and clinical symptoms.

		FSK4				
		+++	++	+	-	Total
IgG EIA	+++	33	28	3	1	65
	++	1	34	2	12	49
	+	0	13	11	18	42
	-	0	0	12	582	594
	Total	34	75	28	613	750

Symbols: +++ Strong positive reaction
++ Moderate positive reaction
+ Weak positive reaction
- Negative reaction

Specificity is high (582/594 = 98.0%) but sensitivity of FSK4 in comparison with EIA is relatively low (125/156 = 80.1%). It is clear, however, from the above data that whilst some EIA positives (31) are not detected by FSK4, there are a number of samples (12) which are FSK4 positive, EIA negative.

REPRODUCIBILITY

Intra-batch reproducibility was established by testing the positive control and antigenic extracts in one batch of product on three separate occasions. One batch of FSK DDP diffusion plates was used throughout. Whilst there was some variation in the number of precipitin lines visualised after staining, a clear positive reaction was seen on each occasion with the positive control and the five antigenic extracts.

Inter-batch reproducibility was examined by testing the positive control and antigenic extracts in three batches of product. A clear positive reaction was seen between the positive control and each of the five antigenic extracts in all batches although there was some variation in the number of precipitin lines generated.

ZWECKBESTIMMUNG

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Das Microgen Bioproducts Vogel-Immundiffusionssystem ist ein Gel-Doppeldiffusions-Assay, der zum Nachweis von als Reaktion auf die Inhalation von Vogelallergenen gebildetem Präzipitin in humanem Serum bestimmt ist. Die mit dem Vorhandensein dieser Antikörper assoziierten Krankheiten werden abhängig von der Expositionsquelle und der von den Untersuchern verwendeten Nomenklatur unterschiedlich als Tauben- oder Vogelzüchterlunge (oder -Krankheit) bezeichnet. Wellensittiche, Truthähne, Hühner und Fasane können eine ähnliche Erkrankung erzeugen, und der Begriff Hypersensitivitätspneumonitis gegen Tauben (oder andere Vögel) ist ebenfalls verwendet worden. Alle diese Krankheiten sind Teil einer Gruppe von Erkrankungen, die exogen-allergische Alveolitis genannt werden.
Das Kit sollte nur von Fachpersonal zu Laborzwecken verwendet werden.

TESTPRINZIP

Das von den Kits nachgewiesene Präzipitin wird als Teil der Immunantwort auf von Tauben, Wellensittichen und Geflügel stammende Antigene erzeugt. Diese Antikörper gehören vorwiegend zur IgG-Klasse, obgleich andere Klassen an der Präzipitation beteiligt sein können.
Der Test beruht auf dem Prinzip der Gel-Doppeldiffusion (Ouchterlony). Wenn lösliche Antigene und homologe Antikörper in benachbarte Vertiefungen gegeben werden, die in geeignete Diffusionsmedien wie Agar oder Agarose gestanzt sind, diffundieren sie aufeinander zu und erzeugen entlang der Grenzflächen der optimalen relativen Konzentrationen sichtbare Präzipitationslinien. Ein polyvalentes Positivkontroll-Antiserum, das geeignete Titer der entsprechenden Antikörper enthält, wird in benachbarten Vertiefungen zu den mit dem Patientenserum reagierenden Antigenen getestet. Die Entwicklung der Präzipitinlinien in den Kontrolltests wird zur Bestätigung der Reagenzien- und Plattenleistung herangezogen. Mit einiger Erfahrung kann die Kontrolle als Anzeige für die Vollendung der Diffusion verwendet werden. Es wird angenommen, dass durch die Verwendung von Vogelserum und Kotextrakten die maximale Anzahl aller positiven Seren identifiziert werden kann. Folglich sollte die Diagnose hypersensitiver Reaktionen beträchtlich erleichtert werden.

CONT

INHALT DES KITS

CONTROL +	FSK4/a	Positivkontrolle: polyvalentes Schaf-Antiserum: 4,0 ml
PIGEON SERUM	FSK4/b	Taubenserum: (vorverdünnt) 0,5ml
BUDGIE SERUM	FSK4/c	Wellensittichserum: (vorverdünnt) 0,5ml
POULTRY SERUM	FSK4/d	Geflügelserum: (vorverdünnt) 0,5ml
PIGEON FEX	FSK4/e	Taubenkotextrakt (gereinigt - 4mg/ml) 0,5ml
BUDGIE FEX	FSK4/f	Wellensittichkotextrakt (gereinigt - 4mg/ml) 0,5ml

Alle Reagenzien enthalten 0,099% Natriumazid als Konservierungsmittel.

Gebrauchsanweisung
Registerkarten (20)

Zusätzlich werden benötigt:

- Doppeldiffusionsplatten (Pakete mit 20 gebrauchsfertigen Platten sind von Microgen Bioproducts unter dem Produktcode FSK/DDP erhältlich)

WARNHINWEISE UND SICHERHEITSVORKEHRUNGEN

Sicherheit:

1. Die Reagenzien in diesem Kit sind nur für die *In-vitro*-Diagnostik gedacht.
2. Natriumazid, das als Konservierungsmittel für die Reagenzien verwendet wird, kann mit in Abflussinstallationen vorhandenem Blei oder Kupfer reagieren und zur Anreicherung von explosiven