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REF

FSK1 ASPERGILLUS IMMUNODIFFUSION SYSTEM

IVD

GB

Instructions for Use
Record Cards (20)

INTENDED USE

The Microgen Bioproducts *Aspergillus* Immunodiffusion System is a serological double gel diffusion assay intended for the detection of precipitating antibodies to *Aspergillus fumigatus* in serum. The kit is intended for professional laboratory use only.

PRINCIPLE OF THE TEST

The precipitating antibodies detected by the kits are generated as part of the immune response to exposure to the antigens of *Aspergillus*. 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. Control sera containing suitable titres of the relevant antibodies are 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 controls can be used as markers indicating completion of diffusion. By utilising two antigen concentrations (20mg/mL and 2mg/mL) and careful selection of the method of preparation of antigenic extracts (1), it is believed that the maximum number of all positive sera will be identified. Consequently, the diagnosis of systemic infection or of hypersensitivity reactions should be made considerably easier. The presence of antibodies resulting from previous exposure to the causative agents can often be differentiated from current infection since the latter is typically characterised by high or rapidly rising titres over a period of time.

KIT PRESENTATION

CONT	

CONTROL	-
CONTROL	+
CF AG	20
CF AG	2
SOM AG	20
SOM AG	2

FSK/a Negative Control: Sheep serum 2.5mL

FSK1/b Positive Control: Sheep anti-*Aspergillus fumigatus* antiserum 4.0mL

FSK1/c *Aspergillus fumigatus* culture filtrate antigens (20mg/mL): 0.5mL

FSK1/d *Aspergillus fumigatus* culture filtrate antigens (2mg/mL): 0.5mL

FSK1/e *Aspergillus fumigatus* somatic antigens (20mg/mL): 0.5mL

FSK1/f *Aspergillus fumigatus* somatic antigens (2mg/mL): 0.5mL

All reagents contain 0.099% sodium azide as a preservative.

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 Aspergillus 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.

The use of citrate eliminates the calcium-dependent reaction between C-reactive protein (CRP) present in some sera and C-substance present in some *Aspergillus* extracts (4).

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 each type of extract selected must be tested separately. The patient's serum is placed in the large well and appropriate control sera in the outer wells. (Sufficient control sera are included to fill 40 x positive control wells and/or 25 x negative control 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 the 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 appear at the 20mg/mL and 2mg/mL antigen levels; these can be used as a semi-quantitative guide to titre. In addition, it is useful to record whether a line is sharp or broad and fuzzy.

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

The determination of precipitins against *A. fumigatus* is extremely useful in aiding the diagnosis of sensitisation to *A. fumigatus* or of saprophytic colonisation by the organism (usually in lung cavities). It has been reported (2,3,5,6) that about 10% of patients with extrinsic asthma have precipitins *to A. fumigatus*. 60% or more of patients with allergic broncho-pulmonary aspergillosis have precipitins to *A. fumigatus*, and patients with aspergilloma almost always have precipitins in the serum (approximately 95%). Patients with extrinsic allergic alveolitis due to Aspergillus may also have precipitins. Rises in precipitin titre can be observed by an increase in the number of precipitin lines, and reduction in the number of lines is an indicator of success of chemotherapy. The number of lines is valuable evidence in diagnosis. One or two bands may indicate any form of aspergillosis but generally this number appears in patients with allergic aspergillosis. Three, four or more precipitin lines is strongly suggestive of either aspergilloma or invasive aspergillosis (3,6).

See Bibliography below for further information on correlation of precipitin line formation with *Aspergillus* infection.

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 Aspergillus.
2. The absence of precipitins does not preclude presence of disease caused by Aspergillus. Early stage infections may only produce very low levels of detectable antibody. Immunosuppressive therapy may result in negative serology. Invasive aspergillosis usually occurs in an immunocompromised host in whom precipitating antibodies may not be produced.
3. The presence of low levels of precipitins may simply indicate past or present exposure to Aspergillus with no incidence of disease.

PERFORMANCE CHARACTERISTICS

There is no reference test procedure for precipitating antibodies with which to compare and evaluate FSK1.

2441 serum samples have been tested with FSK1 and an enzyme immunoassay for IgG to Aspergillus but correlation between the two sets of data is limited due to the following factors:

- Precipitating antibodies reacting in FSK1 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 FSK1.

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

		FSK1				
		+++	++	+	-	Total
IgG EIA	+++	38	33	4	20	95
	++	6	49	13	109	177
	+	3	40	14	266	323
	-	0	20	22	1804	1846
	Total	47	142	53	2199	2441

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

Specificity is very high (1804/1846 = 97.7%) but sensitivity of FSK1 in comparison with EIA is low (200/595 = 33.6%). It is clear, however, from the above data that whilst many EIA positives (395) are not detected by FSK1, there are a number of samples (42) which are FSK1 positive, EIA negative.

REPRODUCIBILITY

Intra-batch reproducibility was established by testing the 4 positive controls and 1 negative control 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 all positive controls. No reactions with the negative control were seen.

Inter-batch reproducibility was examined by testing the 4 positive controls and 1 negative control in three batches of product. A clear positive reaction was seen with all positive controls in all batches although there was some variation in the number of precipitin lines generated in the reaction. No reactions were seen with the negative control.

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ZWECKBESTIMMUNG

Das Microgen Bioproducts *Aspergillus*-Immundiffusionssystem ist ein serologischer Gel-Doppeldiffusions-Assay, der zum Nachweis von Präzipitin gegen *Aspergillus fumigatus* in Serum bestimmt ist. Das Kit sollte nur von Fachpersonal zu Laborzwecken verwendet werden.

TESTPRINZIP

Das von den Kits nachgewiesene Präzipitin wird als Teil der Immunantwort auf die *Aspergillus*-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. Kontrollseren, die geeignete Titer der entsprechenden Antikörper enthalten, werden 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 können die Kontrollen als Anzeige für die Vollendung der Diffusion verwendet werden. Es wird davon ausgegangen, dass durch die Verwendung von zwei Antigenkonzentrationen (20mg/ml und 2mg/ml) und einer sorgfältigen Auswahl der Herstellungsmethode der Antigenauszüge (1) die maximale Anzahl aller positiven Seren identifiziert werden kann. Folglich sollte die Diagnose einer systemischen Infektion oder hypersensitiver Reaktionen beträchtlich erleichtert werden. Das Vorhandensein von Antikörpern, die aus früherem Kontakt mit den auslösenden Krankheitserregern stammen, kann oftmals von einer aktuellen Infektion unterschieden werden, da die letztere typischerweise durch hohe oder über einen gewissen Zeitraum schnell ansteigende Titer gekennzeichnet ist.

CONT	**INHALT DES KITS**

CONTROL	**-**	FSK/a Negativkontrolle: Schafserum 2,5 ml
CONTROL	**+**	FSK1/b Positivkontrolle: Schaf-Anti-*Aspergillus fumigatus*-Antiserum 4,0 ml
CF AG	**20**	FSK1/c *Aspergillus fumigatus*-Kultur-Filtratantigene (20mg/ml): 0,5ml
CF AG	**2**	FSK1/d *Aspergillus fumigatus*-Kultur-Filtratantigene (2mg/ml): 0,5ml
SOM AG	**20**	FSK1/e *Aspergillus fumigatus*-Körperantigene (20mg/ml): 0,5ml
SOM AG	**2**	FSK1/f *Aspergillus fumigatus*-Körperantigen (2mg/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 Metallaziden führen. Bei Entsorgung mit reichlich Wasser nachspülen, um eine Anreicherung des Azids zu vermeiden.
3. Beim Umgang oder der Entsorgung von potenziell pathogenen Proben müssen entsprechende Sicherheitsvorkehrungen getroffen werden. Die Dekontamination infektiösen Materials kann mit Natriumhypochlorit bei einer Endkonzentration von 3 % über 30 Minuten erfolgen. Flüssige Abfallstoffe, die Säuren enthalten, müssen vor der Behandlung neutralisiert werden.
4. Die Antigenpräparte wurden während des Herstellungsprozesses inaktiviert. Trotzdem sollten sie als potenziell infektiös behandelt werden.

Anwendung:

1. Das Kit muss gemäß dieser Gebrauchsanweisung benutzt werden.
2. Während des Gebrauchs dürfen die Reagenzien nur so kurz wie möglich der Umgebungstemperatur ausgesetzt werden.
3. Keines der Reagenzien im Kit darf verdünnt werden.
4. Reagenzien verschiedener Chargen dürfen nicht miteinander vermischt werden.
5. Keines der Reagenzien im Kit darf eingefroren werden.