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ANTI-HUMAN GLOBULIN (Murine Monoclonal)

Anti-C3d

Automated C3d Plate

For Automated Direct Antiglobulin Tests

DOES NOT CONTAIN ANTIBODIES TO IMMUNOGLOBULINS

IVD

Rx Only

30°C
1°C

Immucor, Inc.
3130 Gateway Drive
Norcross, GA 30071 USA
US License No: 0886
MADE IN USA

IMMUCOR

xxxx-1

Intended Use:

Automated C3d Plate is intended for use on Echo Lumena and Galileo Echo in automated direct antiglobulin tests (DAT) where detection of C3d is required.

Summary of the Test:

The principle of the antiglobulin reaction, as described in 1908 by Moreschi [1], was first applied in blood group serology by Coombs and his associates in 1945 [2,3], when the serum of animals immunized with human protein was in the detection of so-called "incomplete" antibodies. The ability of antiglobulin serum to react with components of human complement bound to the red blood cells was reported in 1957 by Dacie and fellow-workers [4]. The antiglobulin (Coombs) test is an important procedure for the detection of immunoglobulin and/or complement bound to the red blood cells. The direct antiglobulin test is used to demonstrate antibodies and/or complement bound to the red blood cells in vivo, and the indirect antiglobulin test is used, after incubation of serum or plasma and red blood cells, to demonstrate antibodies and/or complement bound in vitro.

The following Table summarizes the applications and limitations of different Anti-Human Globulin reagents.

	Anti-IgG,-C3d (polyspecific)	Anti-IgG	Anti-C3b,-C3d (Anti-C3)
Direct Antiglobulin Tests			
Diagnosis of hemolytic disease of the newborn	Yes	Yes	
Investigation of transfusion reactions	Yes	Yes†	Yes†
Investigation of drug-induced red blood cell sensitization	Yes	Yes†	Yes†
Investigation of autoimmune hemolytic anemia	Yes	Yes†	Yes†
Specific identification of cell surface coat (e.g., C3, IgG, IgM, IgA, etc.)		Yes	Yes
Indirect Antiglobulin Tests			
Compatibility testing	Yes	‡	
Screening for unexpected antibodies in donors	Yes	Yes‡	
Screening for unexpected antibodies in patients	Yes	‡	
Identification of antibodies	Yes	Yes	Yes
Detection of antigens	Yes	Yes	

†The red blood cells should be tested for the presence of both IgG and C3d, using either Anti-IgG,-C3d; (polyspecific) or using Anti-Human Globulin reagents that contain these components separately (e.g., Anti-IgG and Anti-C3d).

‡Some literature reports indicate that Anti-IgG occasionally fails to detect antibodies that are demonstrable by means of an Anti-Human Globulin reagent containing an anti-C3 component. Antibodies not detected by Anti-IgG may be clinically significant in some cases.

Principle of the Test:

C3d bound *in vivo* to the red blood cells may be detected by testing the red blood cells with anti-C3d obtained from monoclonal antibodies secreted by hybridoma cells derived from immunizing mice and grown in fluid culture [5]. Anti-C3d is bound on the surfaces of polystyrene microwells. A dilute suspension of the red blood cells is added to the microwell. Centrifugation brings the red blood cells in contact with the anti-C3d. In the case of a positive test, the migration of the red blood cells is impeded by binding to the anti-C3d and the red blood cells adhere to the microwell surface. In a negative test, the red blood cells will not be impeded during their migration and will pellet to the bottom of the microwells as a button of red blood cells.

Reagents:

Key:
Underline = Addition or significant change; ▲ = Deletion of text

Automated C3d Plate consisting of 1x8 strips of microwells containing bound anti-C3d murine monoclonal antibodies secreted by the hybridoma cell line F139 4B4-4. Twelve (12) 1x8 strips are packaged with a support frame and enclosed in a foil pouch to which a desiccant and moisture indicator have been added. Each strip is ready to be used as supplied. Strips can be used singly or in multiples.

The Automated C3d Plate microwells may have residue when the strips are removed from the pouch. This may appear as fluid level lines or cloudiness in the wells. This may occur during the manufacturing process and is not an indication of a product issue.

Warnings and Precautions:

For *in vitro* diagnostic use.
For laboratory professional use.
Strips removed from pouches should be used within eight hours.
Caution: The absence of murine virus has not been established.
Handle and dispose of test wells as if potentially infectious.
Not for use with cord blood specimens.

Storage and Stability:

Store at 1-30 °C when not in use.
Do not freeze or expose to elevated temperatures.
Do not use beyond expiration date which is expressed as CCYY-MM-DD (year-month-day).
If the humidity indicator enclosed within a pouch shows presence of moisture (by the humidity indicator turning from blue to pink), the strips should not be used.
Unused strips, desiccant and humidity indicator should be immediately and carefully resealed within the foil pouch to prevent exposure to moisture.
Strips within resealed pouches should not be used if the humidity indicator shows the presence of moisture.

Specimen Collection and Preparation:

No special preparation of the patient or donor is required prior to specimen collection. Blood should be collected by asceptic technique. Blood should be drawn into EDTA and tested as soon as possible. If delay in testing should occur, the specimen must be stored at 1°– 10 °C. Blood drawn into EDTA should not be stored longer than 7 days. Storage may result in weaker-than-normal reactions.

Triglycerides up to 600 mg/dL and bilirubin up to 30 mg/dL do not interfere with the C3d-DAT assay. Do not use specimens containing 3+ or greater visual hemolysis. Do not use clotted specimens.

Procedure:

Materials Provided

Automated C3d Plate (microwells coated with anti-C3d)

Materials Required But Not Provided

1. Galileo Echo* (Software version 2.1 or higher)
2. Echo Lumena*
3. Phosphate-buffered (approximately 15mM) isotonic saline, pH 6.5-7.5
4. Complement Control Cells (C3d-positive control)
5. Specimen Diluent

*It is the users responsibility to validate an accessory device (either listed or otherwise) for its intended use. Validation results should be maintained as part of the laboratory's records for review by regulatory agencies.

Test Method:

Automated

1. Bring reagents and blood samples to 18-30 °C before testing.
2. Centrifuge the blood samples to separate the plasma/serum from the red blood cells/clot.
3. Remove the desired number of microwell strips from the pouch.
4. Load microwell strips and blood samples onto the instrument following the procedures in the Operator Manual*.
5. Assign the C3d_DAT assay to the blood specimens, either manually or following the upload worklist procedure.
6. Start the C3d_DAT assay following the procedures in the Operator Manual. The instruments automatically perform the C3d_DAT assay, and record and interpret the test results.
   *Galileo Echo/Echo Lumena CHAPTER 3 – INSTRUMENT TESTING OPERATION

Quality Control:

The instrument performs quality control. The Galileo Echo/Echo Lumena includes Complement Control Cells as a positive control with each sample tested.

Interpretation of Results:

Positive Test: Adherence of the red blood cells to part of all of the microwell surface.
Negative Test: No adherence of the red blood cells to the microwell surface.

Limitations:

1. The use of various drugs and certain disease states are known to be associated with a positive direct antiglobulin test.
2. This reagent does not contain antibodies to human immunoglobulins. It is unsuitable for the diagnosis of hemolytic disease of the fetus/newborn.
3. This reagent reacts with red blood cells coated with C3d including intact C3b (C3c/3d) as C3d is a component of C3b.

Specific Performance Characteristics:

Each lot of Automated C3d Plate meets established potency requirements. The murine monoclonal antibodies from hybridoma cell line F139 4B4-4 have been shown to not react with IgG, IgA, IgM or C4. The performance of this product is dependent on adhering to the instructions found in this insert.

Method comparison studies were performed externally at two sites and internally at Immucor, Inc. Specimens were tested with the Automated C3d DAT assay on Galileo Echo/Echo Lumena and a murine monoclonal Anti-C3d comparator reagent; the comparator reagent also detects C3b. Samples with initial equivocal results were retest and the results used for calculations. Discordant specimens were further tested with a murine monoclonal anti-C3d reagent to determine the presence of C3d (Resolved results). Due to the low occurrence of C3-positive red blood cells in the random population, the assay was tested with additional C3d-positive contrived samples. Test results were evaluated for agreement with the comparator methods. Results are summarized in the following tables:

Note: Agreement between methods does not indicate which method is correct.

Initial C3d_DAT 1016 Random Specimens		**Comparator**		**Agreement**	
		Positive	Negative		
Galileo Echo# & Echo Lumena	Positive	10	7	Positive % Agreement	71.43%
				PPA (95% Lower 1-sided CI)	46.00%
	Negative	4	995	Negative % Agreement	99.30%
				NPA (95% Lower 1-sided CI)	98.69%

Resolved C3d_DAT		**Comparator**		**Agreement**	
		Positive	Negative		
Galileo Echo# & Echo Lumena	Positive	10	7	Positive % Agreement	90.91%
				PPA (95% Lower 1-sided CI)	63.56%
	Negative	1	998	Negative % Agreement	99.30%
				NPA (95% Lower 1-sided CI)	98.70%

C3d_DAT 87† Contrived Positive Samples		**Expected Positive**		**Agreement**	
		Positive	Negative		
Galileo Echo# & Echo Lumena	Positive	86	0	Positive % Agreement	100%
				PPA (95% Lower 1-sided CI)	96.58%
	Negative	0	0	Negative % Agreement	N/A
				NPA (95% Lower 1-sided CI)	N/A

†Two samples (2) were initially equivocal. Upon retest, one (1) sample remained equivocal.
#Galileo Echo software version 2.1.

Twenty-nine (29) pediatric specimens were tested with the Anti-C3d assay on Echo Lumena and a murine monoclonal Anti-C3d comparator reagent. The results were compared for agreement. Results also represent assay performance on Galileo Echo software version 2.1.

C3d_DAT 29 Pediatric Specimens		**Comparator**		**Agreement**	
		Positive	Negative		
Echo Lumena	Positive	1	0	Positive % Agreement	100%
				PPA (95% Lower 1-sided CI)	5.00%
	Negative	0	28	Negative % Agreement	100%
				NPA (95% Lower 1-sided CI)	89.85%

For additional information or for technical support (USA/Canada), contact Immucor at 855-IMMUCOR (466-8267).

To get a Certificate of Analysis (CoA) or SDS please go to www.immucor.com and enter Customer Login.

Bibliography:

1. Boral LI, Henry JB. The type and screen: a safe alternative and supplement in selected surgical procedures. Transfusion 1977;17:163.
2. Giblett ER. Blood group alloantibodies: an assessment of some laboratory practices. Transfusion 1977;17:299.
3. Roualt CL. Appropriate pretransfusion testing. In: Pretransfusion testing for the '80s. Washington DC: American Association of Blood Banks, 1980:125.
4. Mollison PL, Engelfriet CP, Contreras M. Blood transfusion in clinical medicine. 9th ed. Oxford: Blackwell Scientific, 1993.
5. Garratty G, Petz LD. The significance of red cell bound complement components in the development of standards and quality control for the anticomplement components of anitglobulin sera. Transfusion 1976;16:297.

Symbols Glossary:


Manufacturer

In vitro diagnostic medical device

Rx Only Prescription Only

Storage temperature limitation (1-30 °C)

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