

ImmunoDOT™

MONO-G TEST



IVD For *In Vitro* Diagnostic Use

INTENDED USE

The Mono-G Test is a qualitative enzyme immunoassay (EIA) that detects IgG antibodies to Epstein-Barr virus capsid antigen (EBV-VCA), Epstein-Barr early nuclear antigen (EBV-EBNA), cytomegalovirus (CMV), and *Toxoplasma*. When used in conjunction with Mono-M Test it is an aid in the serodiagnosis of infectious (EBV) mononucleosis and presumptive serodiagnosis of CMV or *Toxoplasma* mononucleosis-like syndrome.

This assay has not been FDA cleared or approved for the screening of blood or plasma donors. Performance with this device has not been established for either prenatal screening or newborn testing. **Performance of this assay has not been established in a non-clinical laboratory environment (e.g., point of care testing).**

SUMMARY AND EXPLANATION

Mononucleosis is characterized by malaise, fever, hepatosplenomegaly, lymphadenopathy, and abdominal discomfort. Infectious mononucleosis (IM) has multiple etiologies; the most common infectious etiologic agent is Epstein-Barr virus (EBV). Cytomegalovirus (CMV) and *Toxoplasma gondii* infections also may cause mononucleosis syndrome. Mononucleosis caused by EBV is commonly diagnosed serologically by measuring antibodies to the Paul-Bunnell heterophil antigen, but agent specific assays provide more comprehensive laboratory support for diagnosis of mononucleosis syndrome.

ASSAY PRINCIPLE

The assay uses an enzyme-linked immunoassay (EIA) dot technique for the detection of antibodies. Various levels of antigens are provided as discrete dots on the assay strip. When a positive level is obtained, other confirmatory methodologies are recommended. An assay strip is incubated with dilute patient serum, allowing patient antibodies reactive with the test antigens to bind to the solid support membrane. In the second stage, the reaction is enhanced by removal of non-specifically bound materials. During the third stage, alkaline phosphatase-conjugated antihuman antibodies are allowed to react with bound patient antibodies. Finally, the strip is transferred to enzyme substrate reagent which reacts with bound alkaline phosphatase to produce an easily seen, distinct dot.

REAGENTS

Assay Strip. Antigens in order beginning with the window next to the label: Positive reagent control (human serum); EBV-VCA (partially purified EBV lysate from cell strain P3HR1); EBNA-1 (baculovirus origin, purified by affinity chromatography, template B95-8); CMV (glycine lysate of strain AD-169), *Toxoplasma gondii* (purified membrane fraction derived from HEP-2 cell cultured organism purified by column chromatography); and Negative reagent control.

Diluent (#1) Consists of buffered diluent containing protein stabilizers with <0.1% NaN₃.

Enhancer (#2) Consists of sodium chloride with <0.1% NaN₃.

Conjugate (#3) Consists of alkaline phosphatase conjugated goat antihuman IgG (heavy chain specific) in buffered diluent with <0.1% NaN₃.

Developer (#4) Consists of 5-bromo-4-chloro-3-indolyl phosphate and p-nitro blue tetrazolium chloride in buffered diluent with <0.1% NaN₃.

Warnings and Precautions

For *In Vitro* Diagnostic Use. These reagents have been optimized for use as a system. Do not interchange assay strips. Dilution or adulteration of these reagents may also affect the performance of the test. Do not use kit if evidence of microbial contamination is present. Do not use any kits beyond the stated expiration date. Analytic quality deionized or distilled water must be used as Clarifier. Close adherence to the test procedure will assure optimal performance. Do not shorten or lengthen stated incubation times since this may result in poor assay performance.

Some assay components contain sodium azide (NaN₃) that may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup.

Warning - Potential Biohazardous Material: Human sera used in the preparation of this product were tested and found non-reactive for hepatitis B surface antigen and for antibodies to HIV-1, HIV-2, and hepatitis C virus. Because no test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.

Storage

Store reagents and assay strips at 2-8°C. Reagents must be at room temperature (15-30°C) before use. Avoid contamination of reagents.

SPECIMEN COLLECTION AND HANDLING

ImmunoDOT Test is performed on serum. The test requires 10 µL of serum. Lipemic or hemolyzed serum has not been shown to be an acceptable specimen.

Store samples at room temperature for no longer than eight hours. If the assay will not be completed within eight hours, refrigerate the sample at 2-10°C. If the assay or shipment of the samples will not be completed within 48 hours, freeze at -20°C.

PROCEDURE

Materials Provided

Assay Strips	Reaction Vessels
Diluent (#1)	Package Insert
Enhancer (#2)	
Conjugate (#3)	
Developer (#4)	

Materials Required But Not Provided

GenBio Workstation
Specimen collection apparatus
Timer
Analytic quality distilled or deionized water to be used as Clarifier
Pipets
Absorbent toweling to blot dry the assay strip
Positive and Negative Controls (see Quality Control)

Set-Up

1. Turn on Workstation and adjust to appropriate temperature if necessary. Refer to Workstation Instructions.
2. Remove 4 Reaction Vessels per test from the product box and insert into appropriate slots in Workstation. For the large Workstation, add water up to the fill line of the Clarifier Vessel provided. For the small Workstation, use an appropriate container and sufficient water to cover all reactive windows of the assay strip.
3. Place 2 mL Diluent (#1) in Reaction Vessel #1; 2 mL Enhancer (#2) in Reaction Vessel #2; 2 mL Conjugate (#3) in Reaction Vessel #3; and 2 mL Developer (#4) in Reaction Vessel #4.
4. Appropriately label the Assay Strips.
5. If the large Workstation is used, insert the label end of the assay strip into the Strip Holder, one per groove, taking care not to touch the assay windows.

Assay Procedure

1. Add 10 µL serum to Reaction Vessel #1.
2. Prewet Assay Strip by immersing in Clarifier for 30-60 seconds.
3. Using several (5-10) quick up and down motions with the Assay Strip, mix thoroughly in Reaction Vessel #1. Let stand for 60-90 minutes.
4. Remove Assay Strip from Reaction Vessel and swish in the Clarifier. Use a swift back and forth motion for 5-10 seconds allowing for optimal washing of the Assay Strip's membrane windows.
5. Place Assay Strip into Reaction Vessel #2. Mix thoroughly with several (5-10) quick up and down motions. Let stand for 5 minutes.
6. Remove Assay Strip from Reaction Vessel #2 and swish in Clarifier as described (step #4).

7. Place Assay Strip into Reaction Vessel #3. Mix thoroughly with several (5-10) quick up and down motions. Let stand for 30-40 minutes.
8. Remove Assay Strip from Reaction Vessel #3 and swish in Clarifier as described (step #4). DO NOT remove the Assay Strip from the Clarifier.
9. Allow the Assay Strip to stand in the Clarifier for 5 minutes.
10. Remove Assay Strip from Clarifier and place into Reaction Vessel #4. Mix thoroughly with several (5-10) quick up and down motions. Let stand for 5 minutes.
11. Remove Assay Strip from Reaction Vessel #4 and swish in Clarifier as described (step #4).
12. Blot and allow Assay Strip to dry. It is imperative that tests of borderline specimens be interpreted after the Assay Strip has been allowed to dry. **A false positive dot may be identified if the assay strip is not dry when interpreted.**

Reading the Assay Strip

- Positive A dot with an **EASILY SEEN**, distinct border is visible in the center of the window. The outer perimeter of the window must be white to pale gray.
- Negative If no dot is seen or a dot is difficult to see, interpret it as negative. Each window of the assay strip is read independently.

In order to minimize the possibility of "over interpreting" positive test results it is recommended that during initial validation of the assay (as may be required by the laboratory by regulation), the laboratory test a series of presumptive negative samples and each technician interpret the assay strips in a blinded fashion. Please call GenBio Technical Service for further clarification. (To report results, refer to Interpretation Section)

Quality Control

The assay's reagent temperature is between 42-48°C. Due to heat transfer loss, the Workstation temperature is set higher. The appropriate Workstation temperature setting is listed in the Workstation's package insert. (Contact Technical Services for additional guidance if an alternate heat source is used.)

In order to ensure precision at the assay cutoff, a control for each analyte with level(s) falling close to the cutoff is recommended. IgG (Product No. 3908) and IgM (Product No. 3909) positive control sera moderately reactive to all analytes are available. A negative control (Product No. 3920) is also available. NCCLS C24-A should be consulted for guidance on appropriate quality control practices. These should be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Unless otherwise required, it is recommended that control sera be tested upon receipt of a kit. If the control is not reactive, results should not be reported and GenBio Technical Service should be contacted before the kit is used again.

The kit uses reagent controls to assure performance each time a test is performed. The Positive Control window (well #1) contains human serum and tests reagent reactivity. It must be reactive but the intensity must not be used as a calibrator. As a negative reagent control check, the backgrounds around dots and the bottom window (#6) must be white. If the positive is not reactive or the negative is reactive, do not interpret the assay strip.

INTERPRETATION

Negative Interpretation:

Negative results do not rule out the diagnosis of mononucleosis syndrome. The specimen may be drawn before appearance of detectable antibodies. Negative results in suspected early disease should be repeated within 4-6 weeks.

EBV Interpretation:

EBV serodiagnosis uses heterophil, VCA IgM, VCA IgG and EBNA-1 results and classifies the interpretation as current, recent or past infection.

Table 1: EBV Interpretation

Pattern	H1	H2	VCAM	VCAG	EBNA-1	Interpretation
1	+	+	+	+	-	Current Infection
2	+	+	+	-	-	Current Infection
3	+	+	-	-	-	Current Infection
4	+	+	-	+	+	Recent/Past Infection
5	-	-	+	+	+	Recent/Past Infection
6	-	-	-	+	+	Past Infection
7	-	-	-	+	-	Indeterminate [†]

[†] This pattern occurred 5% of the time in prospective studies. Two of the 16 samples were negative; six were from current infections and 8 from past or recent infections as defined by the reference methods. If indicated, it is recommended that a later sample (2-3 weeks) be tested.

Table 2 lists less common reactions. These may occur, but if many or most reaction types are these, please contact Technical Service.

Table 2: EBV Interpretation – Less Common Reactions

Pattern	H1	H2	VCAM	VCAG	EBNA-1	Interpretation
8	-	-	+	+	-	Current Infection
9	+	-	+	+	-	Current Infection
10	+	-	-	+	+	Recent/Past Infection
11	+	-	+	+	+	Recent/Past Infection
12	+	+	+	+	+	Recent/Past Infection
13	-	+	+	+	+	Recent/Past Infection
14	-	-	-	-	+	Past Infection
15	-	+	-	+	+	Past Infection
16	-	+	+	+	-	Indeterminate
17	+	-	-	+	-	Indeterminate [†]
18	-	+	+	-	-	Indeterminate [‡]
19	+	-	-	-	-	Negative

[†] These patterns were not seen in prospective studies and should be cautiously interpreted. If indicated, it is recommended that a later sample (4-6 weeks) be tested.

[‡] These patterns should be infrequent and cautiously interpreted. Please contact Technical Service.

EBV serodiagnosis requires both Mono-M and Mono-G results. Two heterophil antibody levels increase detection probability. The levels should not be used to infer the amount (quantitation) of antibody. Other EBV reaction combinations should not be interpreted. If needed, contact Technical Service.

Due to cross reactivity between EBV and CMV, current or recent EBV infections may be CMV IgM reactive. Using serological methods, it is not possible to determine if CMV infection is concurrent. **Only consider CMV infection if EBV infection is not detected.**

Sera collected for either EBV and/or mononucleosis studies and normal (mononucleosis negative) samples were used to establish cutoffs. The classification by disease stage according to the EBV profile result (heterophil, VCA IgM, VCA IgG, and EBNA-1) was given primary consideration.

CMV Interpretation:

Table 3 details CMV interpretation. This is a presumptive result and further testing may be indicated to assure the serodiagnosis of CMV mononucleosis-like syndrome.

Normal (CMV mononucleosis negative) samples and retrospective CMV IgM positive samples were used to assure the CMV cutoff.

Table 3: Presumptive CMV Interpretation

<u>Stage</u>	<u>Mono-G CMV</u>	<u>Mono-M CMV</u>
Recent or Current	+	+
Past	+	-
Negative	-	-

Toxoplasma Interpretation:

Since the *Toxoplasma* IgG response is typically high during the acute phase, the Mono-G test preferentially detects stronger *Toxoplasma* IgG reactions. If both EBV and CMV results indicate either negative or past infection and *Toxoplasma* IgG is positive, *Toxoplasma* mononucleosis-like syndrome is possible. Thus, a positive Mono-G test should be considered a presumptive result. If clinical and epidemiological information support toxoplasmosis, additional laboratory tests are necessary to assure serodiagnosis.

Normal (*Toxoplasma* mononucleosis negative) samples and retro-spective *Toxoplasma* IgG positive samples were used to assure the *Toxoplasma* cutoff.

LIMITATIONS

- Cross reactivity testing with this assay for antibodies to other closely related organisms and interference testing for RF and/or anti-nuclear antibodies has not been performed.
- ImmunoDOT Mono-M or Mono-G is not designed to assess immune status to EBV, CMV or *Toxoplasma* nor has the test been evaluated in immunosuppressed or immunocompromised individuals.
- Performance in young children (under 12 years old) was not fully evaluated.
- The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy. It is designed for serodiagnosis of IM using the profiles described in the Interpretation section.
- Negative results do not rule out the diagnosis of disease because the specimen may be drawn before appearance of detectable antibodies. However, results in suspected early infection should be repeated within 4-6 weeks.
- Assay performance characteristics have not been established for matrices other than serum. Each physician must interpret these results in light of the patient's history and physical findings.
- Testing should not be performed as a screening procedure for the general population. The predictive value of a positive or negative serologic result depends on the pretest likelihood of infectious mononucleosis (the various analytes included in this assay) being present. Testing should only be done when clinical evidence suggests the diagnosis of infectious mononucleosis syndrome.

EXPECTED RESULTS

ImmunoDOT detects specific antibodies of three organisms that may cause mononucleosis or mononucleosis-like syndrome. EBV is the predominant cause and CMV is the second most frequent cause of mononucleosis. In the U.S., toxoplasmosis causing a mononucleosis-like syndrome is rare.

EBV Mononucleosis

Heterophil: Primary EBV infections in early infancy (less than two years old) are typically unapparent. The heterophil antibody response is barely significant and the infection is silent and shows at most minor hematologic changes^{1,2,3}. The incidence of heterophil antibody responses in the 2 to 5 year old age range is, at most, slightly lower than in adolescents or young adults when sensitive assays are employed. However, the heterophil antibody titers, while covering similar ranges in all age groups, are nevertheless found to increase generally with advancing age. The relatively low heterophil antibody titers of young children explain why such responses may be missed in assays of limited sensitivity, especially rapid slide tests with sheep RBC stromata.

VCA: Sumaya and Ench⁴ report VCA IgM sensitivity at 86% in children under 18 years old. The mean titer was higher in older (4 to 16 years old) than younger (less than 4 years old) children. Lennette⁵ reports that 10-15% of EBV mononucleosis patients have no detectable VCA IgM by the time of the first serum collection, but both IgG and IgM are typically detectable within 2-3 weeks of onset and peak at 4-6 weeks. IgM VCA

disappears rapidly while IgG-VCA wanes slightly and then varies little for life.

EBNA: Anti-EBNA-1 accounts for essentially all of the anti-EBNA in convalescent or later phase sera as measured by the conventional ACIF assay using Raji cells, yet anti-EBNA-2A are the dominant antibodies during the first five months after onset of primary infection⁶.

CMV Mononucleosis

CMV as the cause of mononucleosis has been described since 1969. Friedman reports that CMV is responsible for about 8% of all mononucleosis⁷. Lajo, et.al.⁸ prospectively examined 124 pediatric mononucleosis patients, 12 years old and younger, and reports that 16% of these mononucleosis cases were caused by CMV. Evans⁹ reports that 45% of the cases of CMV mononucleosis occurred over the age of 30 years.

Toxoplasmosis (Mononucleosis-like)

Detection of IgG-specific *Toxoplasma* antibodies is rarely problematic, but low assay specificity of IgM-specific antibodies is reported¹⁰. The incidence of IgG-specific antibody in the U.S. is typically low and the incidence of toxoplasmosis mononucleosis-like syndrome is also rare in the U.S.

Prospective Study Results

Three hundred two samples submitted for mononucleosis serology testing at two laboratories were evaluated. Site A, a national reference laboratory, tested 186 samples and site B, a Midwestern U.S. city hospital laboratory, tested 116 samples. The reference Paul-Bunnell heterophil assay, EBV, CMV and *Toxoplasma* specific serology tests were conducted at an outside reference laboratory. A statistical description of the study populations is shown in Table 4. It should be noted that subjects at the Site A study were younger (median = 17) than subjects at Site B (median = 24). ImmunoDOT performance in this prospective study is shown in the following section (see Performance Characteristics).

Table 4: Population Statistics

<u>Statistic</u>	<u>Site A</u>	<u>Site B</u>	<u>Combined</u>
Number of Samples Tested	186	116	302
Average Age	22.8	27.0	24.4
Median Age	17	24	19
Male/Female ratio	0.73	0.63	0.69
Minimum Age	<1	2	<1
Maximum Age	79	73	79

The age distribution of these cases is shown in Table 5. The average age is 29.8.

Table 5: Age Distribution of EBV IM Cases in Prospective Study

<u>Age Range (years)</u>	<u>Site A</u>	<u>Site B</u>	<u>Combined (%)</u>
>50	1	0	1 (3%)
18-50	8	1	9 (25%)
12-18	14	7	21 (58%)
8-12	1	0	1 (3%)
<8	1	3	4 (11%)

The incidence of ImmunoDOT results at the two sites is shown in Table 6.

Table 6: Prospective Study Results - EBV Infections Only

<u>Classification</u>	<u>Site A</u>	<u>Site B</u>	<u>Combined</u>
Negative	18%	8%	14%
Current	14%	10%	13%
Past/Recent	61%	79%	68%
Indeterminate	7%	3%	5%

ImmunoDOT identified five (12%) additional CMV mononucleosis cases and no toxoplasmosis cases. These observations are consistent with published expectations; however, results in each laboratory may vary due to patient selection criteria, average age of subjects, and various other epidemiological factors.

PERFORMANCE CHARACTERISTICS

A prospective study was performed to assess assay performance. The study and population statistics are described in the previous section,

Expected Results. Site A information based on profile comparison is presented in Table 7. Site B information is shown in Table 8 and the combined data can be seen in Table 9.

Table 7: Site A EBV Performance

<u>ImmunoDOT</u>	<u>Reference Results</u>		
	<u>Negative</u>	<u>Current</u>	<u>Past/Recent</u>
Negative	33	0	1
Current	2	23	1
Past/Recent	0	1	112
Indeterminate	0	6	7

Table 8: Site B EBV Performance

<u>ImmunoDOT</u>	<u>Reference Results</u>		
	<u>Negative</u>	<u>Current</u>	<u>Past/Recent</u>
Negative	9	0	0
Current	1	10	1
Past/Recent	0	0	92
Indeterminate	2	0	1

Table 9: EBV Performance Summary

<u>ImmunoDOT</u>	<u>Reference Results</u>		
	<u>Negative</u>	<u>Current</u>	<u>Past/Recent</u>
Negative	42	0	1
Current	3	33	2
Past/Recent	0	1	204
Indeterminate	2	6	8

There were no *Toxoplasma* IM cases identified during the prospective trial period. Two CMV mononucleosis cases at Site A and three CMV mononucleosis cases at Site B were observed. Three of the five sera from presumptive CMV mononucleosis cases were positive according to reference results. These CMV results are included as ImmunoDOT current positives in Table 10 which summarizes overall ImmunoDOT performance.

Table 10: Overall Performance Summary

<u>ImmunoDOT</u>	<u>Reference Results</u>		
	<u>Negative</u>	<u>Current</u>	<u>Past/Recent</u>
Negative	42	0	1
Current	5	36	2
Past/Recent	0	1	199
Indeterminate	2	6	8

Using the above information, assay performance characteristics are shown in Table 11. Indeterminate results are not used for the calculations.

Table 11: Performance Characteristics

	<u>Sensitivity</u>	<u>Sensitivity Range</u>	<u>Specificity</u>	<u>Specificity Range</u>
EBV Infectious Mononucleosis	99.6% (240/241)	97.7– 100%	93% (42/45)	93-99%
Mononucleosis Syndrome	98.7% (236/239)	96 - 99.7%	89% (42/47)	77-96%

Precision Data

The intensity of the dot is directly related to precision. The darkest dots are most reliable while weaker reactions are proportionately less reliable. Site A and Site B laboratories were supplied masked specimens containing mixtures of the various analytes. Therefore, not all analytes tested the same number of replicates. Testing was conducted in triplicate each day. Tests were performed on six different days. The results (Table 12 and Table 13) show adequate qualitative discrimination for each analyte.

Table 12: ImmunoDOT Mono M Precision Results

<u>Antibody Level</u>	<u>Level 1 Heterophil</u>	<u>Level 2 Heterophil</u>	<u>VCA IgM</u>	<u>CMV IgM</u>
Moderate	100% (36/36)	100% (36/36)	100% (36/36)	100% (72/72)
Low	100% (108/108)	100% (108/108)	100% (72/72)	100% (144/144)

Table 13: ImmunoDOT Mono G Precision Results

<u>Antibody Level</u>	<u>VCA IgG</u>	<u>EBNA IgG</u>	<u>CMV IgG</u>	<u>Toxoplasma IgG§</u>
Moderate	100% (144/144)	100% (36/36)	100% (144/144)	100% (72/72)
Low	100% (72/72)	100% (144/144)	100% (72/72)	85% (122/144)

§ Moderate is greater than or equal to 1:128 IFA titer. Low is less than 1:50 IFA titer.

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Positive Control
EBV-VCA
EBNA-1
CMV
T.gondii
Negative Control

QUICK REFERENCE PROCEDURE

ImmunoDOT Mono G

Set-Up

- Make sure Workstation is at temperature.
- Place reaction Vessels into slots in Workstation and add water to the Clarifier Vessel.
- Place 2 mL Diluent (1) in Vessel #1; 2 mL Enhancer (2) in Vessel #2; 2 mL Conjugate (3) in Vessel #3; and 2 mL Developer (4) in Vessel #4.

Procedure

- Add 10 μ L serum to Vessel #1.
- **Prewet** assay strip in Clarifier for 30 - 60 seconds.
- Place strip in Vessel #1, mix, **let stand 60-90 min.**
- Remove strip, place in Clarifier, swish 5-10 sec.
- Place strip in Vessel #2, mix, **let stand 5 min.**
- Remove strip, place in Clarifier, swish 5-10 sec.
- Place strip in Vessel #3, mix, **let stand 30-40 min.**
- Remove strip, place in Clarifier, **let stand 5 min.**
- Place strip in Vessel #4, mix, **let stand 5 min.**
- Remove strip, place in Clarifier, swish, blot, dry, and read.

To place an order for ImmunoDOT products, contact your local distributor or call GenBio directly for the distributor nearest you and for additional product information.

For assistance, please call toll-free 800-288-4368.



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