CE

DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302
Tel: (818) 591-3030 Fax: (818) 591-8383
onestep@rapidtest.com
technicalsupport@rapidtest.com
www.rapidtest.com

IVD | See external label | 2°C 8°C | Σ 96 tests | REF 1410-1Z

Mumps IgG

Cat # 1410-1Z

Test	Mumps IgG
Method	Enzyme Linked Immunosorbent Assay
Principle	Indirect ELISA : Antigen Coated Plate
Detection Range	Quantitative : Positive, Weak Positive, Negative Control
Sample	10µL
Specificity	96.6%
Sensitivity	99.3%
Total Time	~75 min
Shelf Life	12-14 Months from the manufacturing date

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

INTENDED USE

The Diagnostic Automation Mumps IgG Enzyme linked Immunosorbent Assay (ELISA) is intended for the detection and quantitative determination of IgG antibody to Mumps virus in human sera. Individual serum specimens may be used for the determination of immune status. Paired sera, acute and convalescent, may be used to demonstrate seroconversion or a significant rise in antibody, as an aid in the diagnosis of a recent or current infection. **For *in vitro* diagnostic use. High complexity test.**

Introduction

The mumps virus is a member of the paramyxovirus group and the etiological agent of mumps in man. Mumps is a generalized illness usually accompanied by parotid (salivary gland) swelling and mild symptoms. It is also one of the most common causes of aseptic meningitis, encephalitis, and inflammation of the testes (orchitis), pancreas, and ovaries.

Parotitis as a presenting symptom in mumps infections is usually sufficiently diagnostic to preclude serological confirmation. However, a third of mumps infections are subclinical or unrecognized (1) and may require viral isolation and/or some other serological procedure to confirm or rule out mumps infection. An example of this is presenting orchitis or meningoencephalitis, the two most common complications of mumps infection, without salivary gland involvement. Virus isolation is time consuming and cumbersome and is usually an impractical procedure for the typical clinical laboratory. Current methods for serodiagnosis of mumps infections are in-vitro serum neutralization, hemagglutination-inhibition (HAI), indirect immunofluorescence, and complement fixation (CF) tests. Of these methods, neutralization is reportedly the most specific. However, the neutralization test requires 4-5 days to complete the test. HAI and CF are reportedly less sensitive than the neutralization test.These methods lack specificity, which limits their usefulness in determining immune status. The HAI test also requires pretreatment of test sera to remove nonspecific hemagglutination inhibitors from some sera.

Infection with mumps virus, whether symptomatic or subclinical, is generally thought to offer lifelong immunity.

As first described by Engvall and Perlman (2,3,4) and Van Weeman (5), Enzyme Immunoassays can be both specific and sensitive for the detection and measurement of serum proteins. The sensitivity, specificity, and reproducibility of enzyme-linked immunoassays can be comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination and neutralization (6,7,8,9).

ELISA is as sensitive as the neutralization test and more sensitive than CF and HAI which makes it a reliable test for determination of immune status.

The DAI Mumps IgG ELISA kit provides all the necessary reagents for the rapid determination and quantitation of IgG antibody to mumps virus in human sera.

Principle of the Assay

Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (i.e., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen- antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate, tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H_2SO_4,

the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader (2,3,4,5).

Kit Presentation

Materials Supplied

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

1. **Mumps virus antigen (inactivated) coated microassay plate**: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate; 480T: five plates)
2. **Serum Diluent Type I**: Ready for use. Contains proclin (0.1%) as a preservative. (96T: one bottle, 30 mL, 480T: two bottles, 60 mL each)
3. **Calibrator:** Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature and other testing conditions. (96T: one vial, 0.4 mL; 480T: one vial, 0.8 mL) *
4. **Positive Control**: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Positive Control is utilized to control the positive range of the assay. (96T: one vial, 0.4 mL; 480T: one vial, 0.8 mL) *
5. **Negative Control**: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label.The Negative Control is utilized to control the negative range of the assay. (96T: one vial, 0.4 mL; 480T: one vial, 0.8 mL) *
6. **Horseradish-peroxidase (HRP) Conjugate**: Ready to use. Goat anti-human IgG, containing proclin (0.1%) and gentamicin as preservatives. (96T: one bottle, 16 mL; 480T: five bottles, 16 mL each)
7. **Chromogen/Substrate Solution Type I:** Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 15 mL; 480T: five bottles, 15 mL each)
8. **Wash Buffer Type I (20X concentrate):** Dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-20 and proclin (0.1%) as a preservative. (96T: one bottle, 50 mL; 480T: one bottle, 250 mL)
9. **Stop Solution:** Ready to use, contains a 1N H_2SO_4 solution. (96T: one bottle, 15 mL; 480T: five bottles,15 mL each)

*** Note: serum vials may contain excess volume.**

Additional Requirements

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes (less than 3% CV).
3. One liter graduated cylinder.
4. Paper towels.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water (dH20), CAP (College of American Pathology) Type 1 or equivalent (13, 14).
9. Timer capable of measuring to an accuracy of +/- 1 second (0 – 60 minutes).
10. Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH_20).
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

Note: Use only clean, dry glassware.

Storage and Stability

1. Store unopened kit between 2 and 8°C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2 and 8℃. Unused strips must be immediately resealed in a sealable bag with desiccant and returned to storage between 2 and 8°C.
3. Store HRP Conjugate between 2 and 8°C.
4. Store the Calibrator, Positive Control, and Negative Control between 2 and 8°C.
5. Store Serum Diluent Type I and 20X Wash Buffer Type I between 2 and 8°C.
6. Store the Chromogen/Substrate Solution Type I between 2 and 8°C. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells.
7. Store 1X (diluted) Wash Buffer Type I at room temperature (21 to 25°C) for up to 5 days, or up to one week between 2 and 8℃.

Note: If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to package label for expiration date. Precautions were taken in the manufacture of this product to protect the reagents from contamination and bacteriostatic agents have been added to the liquid reagents. Care should be exercised to protect the reagents in this kit from contamination.

Precautions

1. For in vitro diagnostic use.
2. The human serum components used in the preparation of the Controls and Calibrator in this kit have been tested by an FDA approved method for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen and found negative. Because no test method can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
3. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2 (10).
4. The components in this kit have been quality control tested as a Master Lot unit. Do not mix components from different lot numbers except Chromogen/Substrate Solution Type I, Stop Solution, Wash Buffer Type I, and Serum Diluent Type I. Do not mix with components from other manufacturers.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. All reagents must be at room temperature (21 to 25°C) before running assay. Remove only the volume of reagents that is needed. **Do not pour reagents back into vials as reagent contamination may occur.**
7. Before opening Control and Calibrator vials, tap firmly on the benchtop to ensure that all liquid is at the bottom of the vial.
8. Use only distilled or deionized water and clean glassware.
9. Do not let wells dry during assay; add reagents immediately after completing wash steps.
10. Avoid cross-contamination of reagents. Wash hands before and after handling reagents. Cross-contamination of reagents and/or samples could cause false results.
11. If washing steps are performed manually, wells are to be washed three times. Up to five wash cycles may be necessary if a washing manifold or automated equipment is used.
12. Sodium azide inhibits Conjugate activity. Clean pipette tips must be used for the Conjugate addition so that sodium azide is not carried over from other reagents.
13. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.
14. Never pipette by mouth or allow reagents or patient sample to come into contact with skin. Reagents containing proclin, sodium azide, and TMB may be irritating. Avoid contact with skin and eyes. In case

of contact, flush with plenty of water.
15. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not expose to work area during actual test procedure because of potential interference with enzyme activity.
16. Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with water.
17. **Caution:** Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite (bleach) solution to avoid formation of poisonous gas. Recommend disposing of reacted, stopped plates in biohazard bags. See Precaution 3.
18. The concentrations of anti-Mumps in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

Specimen Collection and Storage

1. Handle all blood, and serum as if capable of transmitting infectious agents.
2. Optimal performance of the DAI ELISA kit depends upon the use of fresh serum samples (clear, non-hemolyzed, nonlipemic, non-icteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture (23). Early separation from the clot prevents hemolysis of serum.
3. Store serum between 2 and 8°C if testing will take place within two days. If specimens are to be kept for longer periods, store at -20°C or colder . Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.
4. If paired sera are to be collected, acute samples should be collected as soon as possible after the onset of symptoms. The second sample should be collected 14 to 21 days after the acute specimen was collected. Both samples must be run in duplicate on the same plate to test for a significant rise. If the first specimen is obtained late during the course of the infection, a significant rise may not be detectable.
5. The NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990) (11).

Methods for Use

Preparation for the Assay

1. All reagents must be removed from refrigeration and allowed to come to room temperature before use (21 to 25°C). Return all reagents to refrige rator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 50 mL of the 20X Wash Buffer Type I to 1 L with distilled and/or deionized H_20. Mix well.

Assay Procedure

Note: To evaluate paired sera, both serum samples must be tested in duplicate and run in the same plate. It is recommended that the serum pairs be run in adjacent wells.

1. Place the desired number of strips into a microwell frame. Allow four (4) Control/Calibrator determinations (one Negative Control, two Calibrators and one Positive Control) per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control/Calibrator configuration. Return unused strips to the sealable bag with desiccant, seal and immediately refrigerate.

Example Configuration:

Plate Location	Sample Description	Plate Location	Sample Description
1A	RB	2A	Patient #4
1B	NC	2B	Patient #5
1C	Cal	2C	Patient #6
1D	Cal	2D	Patient #7
1E	PC	2E	Patient #8 (Acute 1)
1F	Patient #1	2F	Patient #8 (Acute 2)
1G	Patient #2	2G	Patient #8(Convalescent 1)
1H	Patient #3	2H	Patient #8 (Convalescent 2)

RB = **Reagent Blank - Well without serum addition run with all reagents. Utilized to blank reader.**
NC = **Negative Control**
Cal = **Calibrator**
PC = **Positive Control**

2. Dilute test sera, Calibrator and Control sera 1:21 (e.g., 10 µL + 200 µL) in Serum Diluent. Mix well. (For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the patient serum.)
3. To individual wells, add 100 µL of the appropriate diluted Calibrator, Controls and patient sera. Add 100 µL of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.
4. Incubate each well at room temperature (21 to 25°C) for **25 minutes +/- 5 minutes.**
5. Aspirate or shake out liquid from all wells. If using semi- automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out and turn plate upside down and blot on paper toweling to remove all liquid. Repeat the wash procedure two times (for a total of three (3) washes) for manual or semi-automated equipment or four times (for a total of five (5) washes) for automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

****IMPORTANT NOTE**: Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (250-300 µL) is recommended. A total of up to five (5) washes may be necessary with automated equipment. Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.

6. Add 100 µL Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
7. Incubate each well at room temperature (21 to 25°C) for 25 **minutes +/- 5 minutes.**
8. Repeat wash as described in Step 5.
9. Add 100 µL Chromogen/Substrate Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.
10. Incubate each well at room temperature (21 to 25°C) for **10-15 minutes.**
11. Stop reaction by addition of 100 µL of Stop Solution (1N H_2SO_4) following the same order of Chromogen/Substrate addition, including the reagent blank well. Tap the plate gently along the outsides, to mix contents of the wells. The plate may be held up to 1 hour after addition of the Stop Solution before reading.
12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual

wavelength is used, set the reference filter to 600-650 nm. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank is ≥ 0.150 the run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Dispose of used plates after readings have been obtained.

Quality Control

For the assay to be considered valid the following conditions must be met:

1. Calibrator and Controls must be run with each test run.
2. Reagent blank (when read against air blank) must be < 0.150 Absorbance (A) at 450 nm.
3. Negative Control must be ≤ 0.250 A at 450 nm (when read against reagent blank).
4. Each Calibrator must be ≥ 0.250 A at 450 nm (when read against reagent blank).
5. Positive Control must be ≥ 0.500 A at 450 nm (when read against reagent blank).
6. The ISR (Immune Status Ratio) Values for the Positive and Negative Control should be in their respective ranges printed on the vials. If the Control values are not within their respective ranges, the test should be considered invalid and should be repeated.
7. Additional Controls may be tested according to guidelines, or requirements of local, state, and/or federal regulations or accrediting organizations.
8. Refer to NCCLS C24-A for guidance on appropriate QC practices (12).
9. If above criteria are not met upon repeat testing, contact DAI Technical Services.

Interpretation

Calculations

1. Mean Calibrator O.D. (Optical Density) - Calculate the mean O.D. value from the two Calibrator determinations.
2. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined by DAI for each lot of kits. The Correction Factor is printed on the Calibrator vial.
3. Cutoff Calibrator Value - The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Calibrator O.D. determined in Step 1.
4. ISR Value - Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen O.D. Value by the Cutoff Calibrator Value determined in Step 3.

Example:

O.D's obtained for Calibrator = **0.38, 0.42**
Mean O.D. for Calibrator = **0.40**
Correction factor = **0.50**
Cutoff Calibrator Value = **0.50 x 0.40 = 0.20**
O.D. obtained for patient sera = **0.60**
ISR Value = **0.60/0.20 = 3.00**

Analysis

1. The patients' ISR (Immune Status Ratio) values are interpreted as follows:

ISR	Results	Interpretation
≤ 0.90	Negative	No detectable IgG antibody to mumps by the ELISA test. Such individuals are pre- to be uninfected with mumps and to be susceptible to primary infection.

0.91-1.09	Equivocal	Samples should be retested. See number (2) below.
≥ 1.10	Positive	Indicates presence of detectable IgG antibody to mumps by the ELISA test. Indicative of current or previous infection. The individual may be at risk of transmitting mumps infection, but is not necessarily currently contagious.

2. Samples that remain equivocal after repeat testing should be retested on an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken (See Limitations No. 3 and 4).
3. In the evaluation of paired sera, if the acute specimen is negative and the convalescent specimen is positive, a seroconversion has taken place. This indicates a significant change in antibody level and the patient is undergoing a primary infection.
4. To evaluate paired sera for a significant change in antibody level or seroconversion, both samples must be tested in duplicate in the same assay. The mean ISR of both samples (acute and convalescent) must be greater than 1.00 to evaluate the paired sera for significant rise in antibody level.
5. Additional Quality Control for Paired Sera: (See NOTE under Assay Procedure). As a check for acceptable reproducibility of both the acute sera (tested in duplicate) and the convalescent sera (tested in duplicate), the following criteria must be met for valid results:

$$\frac{\text{Acute 1 ISR}}{\text{Acute 2 ISR}} = 0.8 \text{ to } 1.2 \qquad \frac{\text{Convalescent 1 ISR}}{\text{Convalescent 2 ISR}} = 0.8 \text{ to } 1.7$$

6. Compare the ISR of the pairs by calculating as follows:

$$\frac{\text{Mean ISR (second sample)} - \text{Mean ISR (first sample)}}{\text{Mean ISR (first sample)}} \times 100 = \%\ \text{RISE IN ISR LEVEL}$$

% Rise in ISR	Interpretation
< 30.0%	No significant change in antibody level. No evidence of recent infection. If active disease is still suspected, a third sample should be collected and tested in the same assay as the first sample to look for a significant rise in antibody level.
≥ 30.0%	Statistically significant change in antibody level detected. This identifies those persons who are presumed to be experiencing recent or current episodes of mumps infection (reactivation, reinfection or a primary infection where the acute specimen was obtained too late to demonstrate seroconversion.)

Note: When evaluating paired sera, it should be determined if samples with high absorbance values are within linearity specifications of the spectrophotometer. Read the Operator's Manual or contact the instrument's manufacturer to obtain the established linearity specifications of your spectrophotometer.

Expected Values

Mumps is primarily an infection of children with 90% of infections occurring in children younger than 15 years. It is most common during the late winter and early spring. Since many cases are subclinical or unrecognized, many previously infected adults report no history of having mumps, yet the large majority of them, when tested, do have antibodies to mumps (1). Due to the highly contagious nature of mumps, 80 - 90% of susceptible household members will acquire infection.

Limitations of Use

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
2. This kit is designed to measure IgG antibody in patient samples. Positive results in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the fetus before birth. A definitive diagnosis requires viral isolation.
3. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended that an IgM assay be performed, or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion, which is indicative of primary infection.
4. Samples that remain equivocal after repeat testing should be retested by an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken.
5. The results of a single specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.
6. Heterotypic antibodies exist between mumps and parainfluenza virus. Therefore, to confirm the clinical diagnosis of an atypical mumps infection, it is recommended that testing for parainfluenza be done simultaneously to rule out potential cross- reactivity of results.
7. Antibody responses to vaccination is lower than that of a natural mumps infection (1).
8. The values obtained from this assay are intended to be an aid to diagnosis only. Each physician must interpret the results in light of the patient's history, physical findings and other diagnostic procedures.

Performance Characteristics

Sensitivity and Specificity

A total of 190 random samples from three different populations were assayed with the DAI Mumps IgG ELISA and with a second commercially available ELISA test kit. The study population was composed of sera collected from a large metropolitan hospital in the southwestern U.S., a northeastern U.S. Department of Health and randomly collected sera from normal, asymptomatic, ambulatory donors from an upstate New York location. A commercially available Mumps IgG IFA test was used to resolve results which were discordant. The results of this study were as follows:

		DAI ELISA			
		+	-	Relative Sensitivity	Relative Specificity
IFA	+	152	1	99.3	96.6
ELISA	-	1	28	(152/153)	(28/29)

There was complete agreement on one hundred fifty seven (157) samples of which one hundred twenty nine (129) were positive and twenty eight (28) were negative. Thirty three (33) samples gave discordant results and were tested using the referee Mumps IgG IFA test kit. Of the thirty three (33) discordant results, twenty four (24) were positive on the DAI Mumps IgG ELISA and negative on the other ELISA test kit. The referee test (IFA) found twenty three (23) of these discordant samples positive and one (1) negative. Equivocal results (8) by the DAI ELISA were considered indeterminate and omitted from the calculations for relative sensitivity and specificity.

Precision

A study was performed to document typical assay precision with the DAI Mumps IgG ELISA product. The mean, SD, and % CV were calculated for both Intra- and Inter-Assay, and Inter-lot Precision.

Intra-Assay Precision

Table I presents the results of five (5) samples individually pipetted in groups of twenty (20) in a single assay.

TABLE I
Intra-Assay Precision for Mumps IgG

	n	Mean ISR	Std Dev	%CV
Serum 1	20	2.30	0.010	4.8%
Serum 2	20	2.14	0.010	5.6%
Serum 3	20	2.42	0.010	4.1%
Serum 4	20	.20	0.077	38.1%
Serum 5	20	.24	0.054	22.7%

Inter-Assay Precision

Table II presents a summary of the Inter-Assay precision data determined by replicate testing of five (5) samples individually pipetted in groups of five (5) on three (3) consecutive days.

TABLE II
Inter-Assay Precision for Mumps IgG

	Day 1	Day 2	Day 3	n	Mean ISR	Std Dev	%CV
Serum 1	2.33	2.63	2.23	3	2.40	0.200	8.3%
Serum 2	2.21	2.56	2.16	3	2.31	0.220	9.5%
Serum 3	2.32	2.79	2.66	3	0.60	0.260	9.9%
Serum 4	0.32	0.34	0.27	3	0.31	0.034	10.8%
Serum 5	0.30	0.39	0.29	3	0.33	0.058	17.7%

Inter-Lot Precision

Table III presents a summary of the lot to lot precision data determined by the replicate testing of five (5) samples individually pipetted in groups of five (5) using three (3) different lots of reagents.

TABLE III
Inter-Lot Precision for Mumps IgG

	Lot 1	Lot 2	Lot 3	n	Mean ISR	Std Dev	%CV
Serum 1	2.24	2.58	2.40	3	2.41	0.260	10.8%
Serum 2	2.30	2.49	2.41	3	2.40	0.230	9.6%
Serum 3	5.58	2.96	5.58	3	2.71	0.280	10.3%
Serum 4	0.30	0.39	0.33	3	0.35	0.050	14.3%
Serum 5	0.33	0.37	0.36	3	0.36	0.030	8.3%

Intra-Assay Precision for the Percent Rise in ISR Value
Within run precision of serum pairs was determined by testing duplicates of three sera (numbered 1 - 3), five times and using these values to simulate paired sera evaluations for a significant rise in ISR. The results from this study are presented below in Table IV:

TABLE IV

Serum Printing Acute:conv	n	Mean Rise in ISR	SD	%CV	Min.	Max.
1:2	25	29.8%	6.4	21.6%	17.2%	41.5%
1:3	25	185.2%	24.2	13.1%	143.7%	233.0%
2:3	25	119.8%	17.2	14.4%	94.2%	152.0%

Percent Rise in ISR
A study was conducted using actual documented clinical acute and convalescent sera. All sera were tested by the DAI ELISA and complement fixation (CF). Results are presented below in Table V:

TABLE V
Percent Rise in ISR
(Paired Sera Evaluation)

Sample	ISR	% Rise in ISR	CF Titer	Rise in Titer
1A	1.3		<8	
1C	3.3	154 %	64	> 4 FOLD
2A	2.2		8	
2C	3.6	64 %	64	3 FOLD
3A	2.1		8	
3C	12.0	471 %	> 128	> 4 FOLD
4A	1.9		16	
4C	2.6	37 %	64	2 FOLD
5A	2.1		< 8	
5C	6.0	186 %	64	> 4 FOLD
6A	9.1		16	
6C	12.0	32 %	> 128	>3 FOLD
7A	2.3		8	
7C	4.6	100 %	32	2 FOLD

*** (A = acute, C = convalescent)**

Negative = **<** 8
Positive = ≥ 8

Cross-Reactivity

A study was performed to determine the cross-reactivity of the DAI Mumps IgG ELISA with three samples which tested negative by IFA for mumps IgG, and positive for Respiratory Syncytial Virus (RSV) IgG and Antinuclear Antibody (ANA). An additional study was conducted to determine the cross-reactivity of the DAI Mumps IgG ELISA with three samples which tested positive by IFA for parainfluenza virus type 3 (PIV 3) IgG and negative by IFA for mumps IgG. Negative DAI Mumps IgG ELISA test results for all six samples indicate an absence of cross-reactivity of the DAI Mumps IgG ELISA with RSV, ANA and PIV 3.

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DIAGNOSTIC AUTOMATION, INC.
23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302
Tel: (818) 591-3030 Fax: (818) 591-8383

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