CMV IgM EIA ID: Grey

Enzyme Immunoassay (EIA) for the Detection of CMV IgM Antibodies in Human Serum.

For *In Vitro* Diagnostic Use Only 25178 • 96 Tests

CONTENTS

1 - INTENDED USE

For the qualitative detection of human IgM antibodies to cytomegalovirus (CMV) in human serum, by enzyme immunoassay, to aid in the diagnosis of CMV infection. A positive result is presumptive for the detection of anti-CMV IgM antibodies and presumptive for the diagnosis of acute or recent CMV infection. These reagents have not received FDA clearance for use in testing blood or plasma donors.

2 - SUMMARY AND EXPLANATION OF THE TEST

Cytomegalovirus (CMV) has been identified as a major causative agent of congenital abnormalities, including mental retardation and deafness in infants infected *in utero*.[1,2,3] Subclinical infection may occur in adults as well as overt disease including hepatitis, pneumonitis, and cytomegalovirus induced mononucleosis.[4] CMV infection can be transmitted to immunodeficient or immunosuppressed individuals as a result of blood transfusion[5] or organ transplantation.[6] In summary, pregnant women, neonates, and immunocompromised individuals are at risk of developing clinically significant disease caused by CMV infection.

The diagnosis of CMV infection is frequently assisted by serological methods. The demonstration of CMV IgM antibodies is indicative of recent or current infection or, in the case of newborns, of congenital infection.

The CMV IgM EIA test is intended for the detection of IgM antibodies to CMV. Test results are obtained after one and one-half hours incubation time. They are objective and normalized as Index values, permitting uniformity of reporting.

Because enzyme immunoassays for IgM antibodies are performed with unfractionated serum, there are two potential sources of error. These include possible competition by CMV-specific IgG, leading to false negative results; and rheumatoid factor in the presence of CMV-specific IgG, leading to false positive results. The CMV IgM EIA test has been designed to minimize the likelihood of errors due to these causes.

3 - PRINCIPLE OF THE TEST

Diluted samples are incubated in antigen-coated wells. *Absorbents have been included in the Diluent to neutralize the effects of rheumatoid factor and anti-CMV IgG antibody.* CMV antibodies (if present) are immobilized in the wells. Residual sample is eliminated by washing and draining, and conjugate (enzyme-labeled antibodies to human IgM) is added and

incubated. If IgM antibodies to CMV are present, the conjugate will be immobilized in the wells. Residual conjugate is eliminated by washing and draining, and the enzyme-labeled substrate is added and incubated. In the presence of the enzyme, the substrate is converted to a yellow end product which is read photometrically.

4 - REAGENTS

CMV IgM EIA Product Description
Catalog No. 25178 (96 Tests)

Component	Contents	Preparation
Coated Wells 12 eight-well strips	• Coated with CMV antigen, Strain: AD 169, partially purified from nuclear extracts of MRC-5 cells • Grey wells	Use as supplied. Return unused strips to pouch and reseal. Do not remove desiccant.*
Well support 1 Frame	• Plate frame	Use as supplied.
Diluent** 1 bottle (25 mL)	• Phosphate-buffered saline with a protein stabilizer, and absorbents for rheumatoid factor and human IgG • Pink Color	Use as supplied.
Calibrator 1** 1 vial (0.6 mL)	• Human serum, strongly reactive for CMV IgM antibodies • Index value shown on vial label	Dilute in Diluent as described.
Calibrator 2** 1 vial (0.6 mL)	• Human serum, moderately reactive for CMV IgM antibodies • Index value shown on vial label	Dilute in Diluent as described.
Positive Control** 1 vial (0.6 mL)	• Human serum, reactive for CMV IgM antibodies • Index values shown on vial label	Dilute in Diluent as described.
Negative Control** 1 vial (0.6 mL)	• Human serum, non-reactive for CMV IgM antibodies	Dilute in Diluent as described.
Conjugate** 2 bottles (12 mL)	• Goat anti-human IgM labeled with alkaline phosphatase (calf) • Green Color	Use as supplied.
Substrate*** 1 bottle (12 mL)	• p-Nitrophenyl phosphate	Use as supplied.
Wash Concentrate** 1 bottle (30 mL)	• Tris-buffered saline • Tween 20™ • pH 8.0	Dilute in 1 liter of distilled or deionized water.
Stop Reagent 1 bottle (12 mL)	• Trisodium phosphate 0.5 M	Use as supplied.

* The color of the desiccant does not affect the performance of the kit.
** Contains 0.1% sodium azide.
*** The substrate may develop a slight yellow color during storage. One hundred microliters of substrate should yield an absorbance value less than 0.35, when read in a microwell against air or water.

Store these reagents at 2-8°C up to the expiration date indicated on the bottle labels. Do not allow them to contact the skin or eyes. If contact occurs, wash with copious amounts of water. Do not remove desiccant.

5 - OTHER MATERIALS REQUIRED

1. Microplate washer
2. Pipettors for dispensing 8, 100, and 200 µL
3. Timer
4. 1 or 2 liter container for Wash Solution
5. Distilled or deionized water
6. Dilution tubes or microwells
7. Microwell reader capable of reading absorbance at 405 nm.

6 - PRECAUTIONS FOR USERS

For *In Vitro* Diagnostic Use

1. Test samples, Calibrator(s), Controls, and the materials that contact them should be handled as potential biohazards. The calibrators and controls have been tested and found to be non-reactive for HIV, hepatitis B surface antigen, and HCV antibodies by licensed tests. However, no method can offer complete assurance that HIV, hepatitis B virus, HCV, or other infectious agents are absent. Handle reagents and patient samples as if capable of transmitting infectious disease following recommended *Universal Precautions* for bloodborne pathogens as defined by OSHA[9], Biosafety Level 2 guidelines from the current CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*[10], WHO *Laboratory Biosafety Manual*[11], and/or local, regional, and national regulations.
2. The concentrations of anti-CMV IgM in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.
3. Avoid contact with open skin.
4. Never pipet by mouth.
5. Certain test reagents contain dilute **sodium azide**, which may be harmful if enough is ingested (more than supplied in kit). Azides are reported to react with lead and copper in plumbing to form compounds that may detonate on percussion. If disposing of solutions containing sodium azide down drains, flush with large volumes of water to minimize the build-up of metal-azide compounds. Dispose of contents and container in accordance with local, regional, national, and international regulations.

6. For more hazard information, refer to the product Safety Data Sheet (SDS), which is available at www.bio-rad.com and upon request.
7. Any lot number of the following reagents may be used with this assay, provided they have the correct catalog number and are not used beyond their labeled expiration date:
   - Diluent - Catalog # 25188
   - Substrate - Catalog # 25192
   - Wash Concentrate - Catalog # 25190
   - Stop Reagent - Catalog # 25191

   Do not mix any other reagents from different lots.
8. Do not use reagents beyond their stated expiration date.
9. Incubation times recommended in the Test Procedure section should be adhered to.
10. Unused Coated Wells should be kept in their resealable bag with desiccant and stored in the refrigerator.
11. This product should be used by qualified personnel.
12. There are no health hazards associated with the intact desiccant packet. Do not cut, split, or otherwise compromise it as dusts that may be generated could pose a health hazard. If the desiccant has been compromised, do not remove it from the plate pouch.

7 - SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Sera should be separated from clotted blood. If specimens are not tested within 8 hours, they should be stored at 2 to 8°C for up to 48 hours. Beyond 48 hours, specimens should be stored at -20°C or below. Multiple freeze-thaw cycles should be avoided. Samples containing visible particulate matter should be clarified by centrifugation; and hemolyzed, icteric, or grossly contaminated samples should not be used. Samples should not be heat-inactivated before testing.

8 - TEST PROCEDURE

Materials Provided

See REAGENTS section on page 4.

EIA Procedure

1. Allow all reagents and patient samples to reach room temperature before use. Return them promptly to refrigerator after use.
2. Prepare working wash solution by adding entire bottle of Wash Concentrate (30 mL) to 1 liter of water. Once diluted, the wash solution can be stored at room temperature for up to two months, or at 4°C until the expiration date printed on the Wash Concentrate bottle.
3. Prepare 1:26 dilutions of test samples, Calibrator(s), Positive and Negative Controls in the test set Diluent. For example: add 8 µL of sample to 200 µL of Diluent in a dilution well or tube and mix well.

   Note: A single Calibrator (Calibrator 2) may be used; or Calibrator 1 and Calibrator 2 may be used to prepare a calibration curve. The blank serves as the zero calibrator.
4. Place appropriate number of Coated Wells in the Well Support.

   Note: For combination testing (multiple assays per plate), the strips should be assembled on a white background with good lighting. Be sure to note the placement of each strip and the corresponding color.
5. Transfer 100 µL of each diluted Calibrator, Control, and patient sample to the wells.

   Note: Include one well which contains 100 µL of Diluent only. This will serve as the reagent blank and will ultimately be used to zero the photometer before reading the test results.
6. Incubate the wells at room temperature (20 to 25°C) for 30 ± 5 minutes.
7. Wash wells four times, with at least 250 µL of wash solution per well. Do not allow the wells to soak between washes. Aspirate thoroughly after the last wash.
8. Place 2 drops (or 100 µL) of Conjugate into each well.
9. Incubate the wells at room temperature (20 to 25°C) for 30 ± 5 minutes.

10. Wash wells four times with at least 250 µL of wash solution per well. Do not allow the wells to soak between washes. Aspirate thoroughly after the last wash.
11. Place 2 drops (or 100 µL) of Substrate into each well.
12. Incubate at room temperature (20 to 25°C) for 30 ± 5 minutes.
13. Place 2 drops (or 100 µL) of Stop Reagent into each well. Tap the plate gently, or use other means to assure complete mixing.
14. Read and record the absorbance of the contents of each well at 405 nm against the reagent blank.

    Note: Adjust the photometer to zero absorbance at 405 nm against the reagent blank. Readings should be made within 2 hours after the reactions have been stopped.

9 - QUALITY CONTROL

Test Validation Criteria

1. The Calibrator(s), Positive and Negative Controls must be included in each test run.
2. The absorbance value of Calibrator 1 must be ≥ 0.400 when read against the reagent blank.
3. The absorbance value of Calibrator 2 must be ≥ 0.200 when read against the reagent blank.
4. The absorbance value of the reagent blank should be < 0.350.
5. The Negative Control must have an Index value < 0.9.
6. The Positive Control must have an Index value equal to or greater than 1.1 when using a single Calibrator (Calibrator 2). When using the calibration curve, the Positive control must have an Index value within the range printed on the label. Users may supply an alternative Positive Control if they wish.
7. The Negative and Positive Controls are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cutoff. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. For guidance on appropriate quality control practices, please refer to NCCLS document C24-A, *Internal Quality Control Testing: Principles and Definitions*.

8. If any of these criteria are not met, the test is invalid and should be repeated. If the test is invalid, patient results can not be reported.

10-INTERPRETATION OF RESULTS

Calculation of Results

Test results may be calculated using a single calibrator (Calibrator 2), or using a calibration curve.

Single Calibrator (Calibrator 2)
Determine the Index value for each test sample (or Control) using the following formula:

$$\frac{\text{Calibrator 2 Index}}{\text{Calibrator 2 Absorbance}} \times \text{Test Sample Absorbance} = \text{Test Sample Index}$$

If the Calibrator is run in duplicate, use the average absorbance value to calculate results.

Calibration Curve
Alternatively, test results may be calculated from a three-point curve comprised of: Calibrator 1 (high-point), Calibrator 2 (mid-point), and the reagent blank (zero/origin), using a point-to-point curve fit.

Interpretation of Results

Index Value	Interpretation
< 0.9	Negative for anti-CMV IgM antibody
≥ 0.9 and < 1.1	Equivocal
≥ 1.1	Positive for anti-CMV IgM antibody

*Index values which fall between 0.9 and 1.1 indicate an equivocal result. Subsequent samples should be drawn at least fourteen days later and tested simultaneously with the initial sample. If the subsequent sample is positive, seroconversion has occurred, which may be indicative of recent infection. If the subsequent sample remains equivocal, antibody status is undetermined and the sample is deemed equivocal. Other clinical and serological evidence should be sought in these cases.

The presence of IgM antibody to CMV suggests recent or current infection.

The CMV IgM EIA cutoff values were based on statistical analyses, i.e., mean + 3 standard deviations[8], of 78 normal

serum specimens. They were challenged in tests of positive and negative specimens (see Performance Characteristics).

Specimens which yield absorbance values above the range of the test set calibrator(s) may be pre-diluted in the test set Diluent and reassayed. The resulting Index value must be multiplied by the dilution factor. Example: If the specimen has been pre-diluted 1:5 before testing, the resulting Index value should be multiplied by 5.

Values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported IgM level cannot be correlated to an endpoint titer. The magnitude of the assay result above the cutoff is not an indicator of the total antibody present.

Specimens collected too early during the course of the disease may not contain anti-CMV IgM antibody. Furthermore, some individuals may not produce a detectable IgM response to CMV infection.

11-LIMITATIONS

CMV IgM test results are intended as an aid to the diagnosis of active or recent infection and should not be considered diagnostic by themselves. They should be interpreted in conjunction with other clinical findings and diagnostic procedures.

This assay is not intended for viral isolation and/or identification.

The presence of IgM antibodies to CMV is considered to be presumptive evidence of primary infection; however, specific IgM has been reported in reactivation of latent infection and in reinfection. CMV IgM may remain detectable for as long as nine months in immunocompetent individuals, and for up to two years in immunosuppressed individuals.

Up to thirty percent of patients with heterophile antibody positive mononucleosis have been reported to have heterotypic CMV IgM responses. This may be due to polyclonal stimulation of B lymphocytes by Epstein-Barr virus. It has been reported that varicella zoster virus may also cause heterotypic CMV IgM responses.

Rheumatoid factor in the presence of specific IgG may contribute to false positive results. The absorbent in the CMV IgM EIA Diluent is intended to neutralize the effects of rheumatoid factor. Studies have indicated that the absorbent was able to neutralize up to ninety-eight percent of the activity in

a sample known to contain 3,328 IU/mL of rheumatoid factor activity.

The presence or absence of CMV IgG or IgM in pregnant women is of limited value in predicting congenital infection. The presence of CMV IgM in the circulation of newborns, however, is indicative of congenital infection. Samples obtained early in the course of infection may not demonstrate IgM antibody. Therefore, it may be necessary to test another sample obtained seven to fourteen days later. When collecting cord blood, care should be taken to avoid contamination with maternal blood. It is advisable to confirm positive results in newborns with a follow-up test.

The assay's performance characteristics have not been established for testing newborn specimens, or cord blood, or matrices other than human serum.

The assay's performance characteristics were not established for visual result determination.

The IgM response to CMV infection may vary according to the individual. It has been reported that ten to thirty percent of infants infected congenitally may fail to develop CMV IgM antibody responses. In addition, up to twenty-seven percent of adults with primary CMV infection may not demonstrate an IgM response. Therefore, the absence of demonstrable CMV specific IgM does not necessarily rule out the possibility of active or recent CMV infection.

It has been suggested that samples containing high levels of CMV specific IgG, and low levels of CMV specific IgM, may yield false negative results. These conditions may exist in the sera of congenitally infected newborns due to the presence of maternal IgG.

12-EXPECTED VALUES

Studies performed with specimens obtained in the U.S. and in the United Kingdom using the CMV IgM EIA test, revealed the following: The incidence of anti-CMV IgM antibody among normal, asymptomatic donors was 2 of 86, or 2.3%. Among patients whose sera were submitted to clinical laboratories for diagnostic testing, the incidence was 45 of 78, or 57.7%. These results are tabulated in the tables below.

Table 1a: Results of CMV IgM EIA tests of 45 archival specimens (frozen), from normal asymptomatic South Florida blood donors. The assays were performed at Laboratory C, Miami, FL.

Index Value Ranges	Specimens	
< 1.1	43	95.6%
≥ 1.1 to < 2.2	2	4.4%
≥ 2.2 to < 4.4	0	0%
≥ 4.4	0	0%

Table 1b: Results of CMV IgM EIA tests of 41 archival specimens (frozen), from normal asymptomatic donors obtained at Oxford, England. The assays were performed at Lab B, Oxford, England.

Index Value Ranges	Specimens	
< 1.1	41	100%
≥ 1.1 to < 2.2	0	0%
≥ 2.2 to < 4.4	0	0%
≥ 4.4	0	0%

Table 2a: Results of CMV IgM EIA tests of 16 archival Specimens (frozen), from patients whose sera were submitted to a clinical laboratory in Tucker, GA, for diagnostic testing. The assays were performed at Lab C, Miami, FL.

Index Value Ranges	Specimens	
< 1.1	5	31.2%
≥ 1.1 to < 2.2	2	12.5%
≥ 2.2 to < 4.4	6	37.5%
≥ 4.4	3	18.8%

Table 2b: Results of CMV IgM EIA tests of 32 archival specimens (frozen), from patients whose sera were submitted to a clinical laboratory in Oxford, England, for diagnostic testing. At least 14 of the Patients were females of childbearing age. The assays were performed at Lab C, Miami, FL.

Index Value Ranges	Specimens	
< 1.1	23 {9}	71.9%
≥ 1.1 to < 2.2	6 {3}	18.8%
≥ 2.2 to < 4.4	1 {1}	3.1%
≥ 4.4	2 {1}	6.2%

{ } Females of childbearing age.

Table 2c: Results of CMV IgM EIA tests of 30 archival specimens (frozen), from patients whose sera were submitted to a clinical laboratory in Oxford, England, for diagnostic testing. At least 10 of the patients were females of childbearing age. The assays were performed at a public health laboratory (Lab B), Oxford, England.

Index Value Ranges	Specimens	
< 1.1	5 {3}	16.7%
≥ 1.1 to < 2.2	7 {1}	23.3%
≥ 2.2 to < 4.4	18 {6}	60.0%
≥ 4.4	0	0%

{ } Females of childbearing age.

13-PERFORMANCE CHARACTERISTICS

Comparative Testing

The results of CMV IgM EIA tests correlate well with other commercial serological tests. Serum specimens obtained from asymptomatic normal donors and patients whose sera were submitted to clinical laboratories for CMV IgM screening or diagnostic testing were assayed by the CMV IgM EIA test and another commercial serological assay. The assays were performed at two independent laboratories (Lab A, Miami, FL and Lab B, Oxford, England) and at Laboratory C, Miami, FL. The results obtained in these studies are shown below in Tables 3, 4, and 5, respectively.

Table 3: Results of tests of 60 archival patient specimens tested at Laboratory A Miami, FL, using the CMV IgM EIA test and another commercial test.

Comparative Test #2	CMV IgM EIA		
	Positive	Negative	Equivocal
Positive	19 {3}	2	2 {1}
Negative	1	35 {10}	1

	%	95% C.I.**
Relative sensitivity*	90.5	77.9 to 100
Relative specificity*	97.2	91.9 to 100
Overall Agreement*	94.7	88.9 to 100

* Excluding equivocal results

** Calculated by the Normal Method[7]

{ } Females of childbearing age.

Table 4: Results of tests of 71 archival patient specimens tested at a public health laboratory, Oxford, England (Laboratory B), using the CMV IgM EIA test and another commercial test.

Comparative Test #1	CMV IgM EIA		
	Positive	Negative	Equivocal
Positive	25 {7}	0	2 {1}
Negative	0	44 {2}	0

	%	95% C.I.**
Relative sensitivity*	100	88.8 to 100
Relative specificity*	100	93.5 to 100
Overall Agreement*	100	95.8 to 100

* Excluding equivocal results
** Calculated by the Normal Method[7]
{ } Females of childbearing age.

Table 5: Results of tests of 121 archival patient specimens tested at Laboratory C, Miami, FL, using the CMV IgM EIA test and another commercial test.

Comparative Test #1	CMV IgM EIA		
	Positive	Negative	Equivocal
Positive	38 {5}	1 {1}	4 {1}
Negative	0	78 {7}	0

	%	95% C.I.**
Relative sensitivity*	97.4	92.5 to 100
Relative specificity*	100	98.7 to 100
Overall Agreement*	99.1	97.5 to 100

* Excluding equivocal results
** Calculated by the Normal Method[7]
{ } Females of childbearing age.

Please be advised that "relative" sensitivity and specificity refers to the comparison of this assay's results to that of a similar assay. No judgment can be made on the comparison assay's accuracy to predict disease.

Cross-reactivity

Of fifty-three specimens which were unreactive in the CMV IgM EIA test, 5 were shown to contain moderate to high levels of IgM antibody directed against rubella virus, 5 against varicella zoster virus, 8 against Epstein-Barr virus (VCA), 16 against herpes simplex virus, 5 against *Toxoplasma*, 10 against type A influenza virus, 10 against measles, and 10 against parvovirus.

IgM Specificity Study

Seven serum specimens which contained CMV-specific IgM and CMV-specific IgG were assayed by the CMV IgM EIA and CMV IgG tests before and after treatment with 2-mercaptoethanol.

This treatment denatures IgM but does not affect IgG antibodies. The results of this experiment are shown in Table 6, below.

Table 6: Results obtained for CMV IgM EIA and CMV IgG EIA assays of seven serum specimens containing CMV-specific IgG and IgM, before and after treatment with 2-mercaptoethanol.

	CMV IgG (Index)		CMV IgM (Index)	
Sample	Before	After	Before	After
1	4.9	4.4	1.1	0.1
2	7.8	7.1	1.6	0.1
3	11.0	9.8	1.5	0.1
4	9.8	10.4	4.1	0.2
5	5.3	4.6	1.7	0.1
6	4.6	5.0	1.9	0.1
7	2.1	1.9	2.4	0.0

After treatment with 2-mercaptoethanol, the CMV IgM antibodies in all seven specimens were neutralized, while the CMV IgG antibodies were not significantly affected. These results demonstrate that the CMV IgM EIA test is specific for detecting CMV IgM antibodies.

Precision

Eight serum specimens (2 negative and 6 positive) and the CMV IgM EIA positive and negative controls were assayed in triplicate on three separate occasions.

The precision experiments were performed manually at two independent laboratories (Lab A and Lab B) and at Lab C. These results are shown below in Tables 7, 8, and 9, respectively.

Table 7: Results of intra-assay and inter-assay precision tests performed at Lab A. Values were calculated from CMV IgM EIA Index values.

	INTRA-ASSAY			INTER-ASSAY		
SAMPLE	MEAN INDEX	S.D.	C.V. %	MEAN INDEX	S.D.	C.V. %
Pos. Control	1.6	0.138	8.7	1.8	0.150	8.5
Neg. Control	0.0	0.000	NA	0.0	0.008	NA
1	0.0	0.015	NA	0.0	0.026	NA
2	0.0	0.000	NA	0.0	0.001	NA
3	2.0	0.043	2.2	2.1	0.141	6.6
4	1.6	0.085	5.4	1.8	0.194	10.9
5	1.3	0.098	7.5	1.5	0.187	12.4
6	1.2	0.071	5.8	1.4	0.172	12.1
7	1.6	0.196	12.2	1.8	0.211	11.6
8	1.1	0.138	12.2	1.4	0.212	15.5

Table 8: Results of intra-assay and inter-assay precision tests performed at Lab B. Values were calculated from CMV IgM EIA Index values.

	INTRA-ASSAY			INTER-ASSAY		
SAMPLE	MEAN INDEX	S.D.	C.V. %	MEAN INDEX	S.D.	C.V. %
Pos. Control	1.7	NA	NA	1.9	0.138	7.4
Neg. Control	0.0	NA	NA	0.0	0.004	NA
1	0.0	0.018	NA	0.1	0.021	NA
2	0.0	0.000	NA	0.0	0.039	NA
3	2.0	0.158	7.9	2.3	0.253	10.9
4	1.7	0.113	6.5	2.0	0.259	12.9
5	1.1	0.132	11.7	1.4	0.231	17.0
6	1.2	0.093	7.7	1.3	0.147	11.1
7	1.5	0.111	7.2	1.7	0.202	11.6
8	1.0	0.085	8.2	1.3	0.196	15.3

Table 9: Results of intra-assay and inter-assay precision tests performed at Lab C. Values were calculated from CMV IgM EIA Index values.

	INTRA-ASSAY			INTER-ASSAY		
SAMPLE	MEAN INDEX	S.D.	C.V. %	MEAN INDEX	S.D.	C.V. %
Pos. Control	1.7	0.058	3.5	1.7	0.101	5.8
Neg. Control	0.0	0.058	NA	0.0	0.033	NA
1	0.1	0.000	NA	0.1	0.000	NA
2	0.0	0.000	NA	0.0	0.000	NA
3	2.0	0.153	7.5	2.1	0.109	5.1
4	1.7	0.058	3.3	1.9	0.001	5.5
5	1.5	0.058	3.9	1.6	0.088	5.7
6	1.5	0.058	3.8	1.7	0.113	6.8
7	1.7	0.115	6.7	1.9	0.141	7.6
8	1.5	0.058	3.9	1.6	0.101	6.5

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