SUPPLEMENTAL MATERIAL

Supplementary Material and Methods

MeV-specific PCR and genotyping

For the PCR analyses, viral nucleic acid was extracted from 200 µl of clinical sample using the automated NucliSens EasyMag extractor, according to the manufacturer´s instructions (Biomerieux, Marcy l´Etoile, France). For the detection of MeV RNA, a primer and probe mix (LightMix®Modular Measles Kit, Tib-MolBiol, Berlin, Germany) was used (1). The PCR assays were validated using proficiency panels from QCMD (Quality Control for Molecular Diagnostics, Glasgow, United Kingdom), NEQAS (National External Quality Assessment Service, Sheffield, United Kingdom) and Instand (Instand, Düsseldorf, Germany). All PCR positive samples were genotyped, confirming infection with the wild virus (2).

IgG and avidity testing

EUROIMMUN Anti-Measles Virus IgG ELISA (Euroimmun, Lübeck, Germany) was used for quantification of MeV IgG antibodies and performed according to the manufacturers' instructions (3). Results were classified as negative if IgG titer was <200 IU/L, 200-275 IU/L as borderline and >275 IU/L as positive. Additionally, MeV IgG avidity was measured using the above-mentioned IgG ELISA (Euroimmun, Lübeck, Germany) according to the manufacturers' instructions. Low avidity, borderline avidity and high avidity were defined as avidity <40%, 40-60% and >60%, respectively.

Neutralization test (NT)

An in-house NT was used to assess MeV neutralizing antibodies. In brief, two-fold serial dilutions of heat-inactivated serum samples were incubated with 50-100 TCID50 MeV strain B3 for 1h at 37 °C before the mixture was added to confluent Vero-SLAM cell monolayers. Incubation was continued for four days. The presence of virus in the supernatant was assessed by the occurrence of cytopathic effects. NT titers ≥ 10 were considered positive. The NT was calibrated using the World Health Organization (WHO) 3rd International Standard Anti-Measles Serum (National Institute for Biological Standards and Control, NIBSC, Code 97/648), and an NT titer of 10 is equivalent to a concentration of 120 mIU/ml.

IgM antibody testing for other viral infections in control samples

All 153 samples from controls without MeV infection (group= 3) were tested for Epstein Barr Virus (EBV)- and Parvovirus B19 (B19)-specific IgM antibodies using the Euroimmun Anti-EBV-CA-IgM ELISA and the Anti-B19V-IgM ELISA (both Euroimmun, Lübeck, Germany) according to the manufacturers´ instructions using the recommended cutoffs.

Ethics

The study was approved by the ethics committee of the Medical University of Vienna (EK 2156/2019) and performed in accordance with the Declaration of Helsinki (2013). Since the samples had been acquired for virological diagnosis and had already been anonymized when they were integrated into the sample bank of the Center of Virology for research, the local ethics committee concluded that no written informed consent from the patients was required (EK 1035/2016, EK 1513/2016).

Supplementary Table S1. Serological profiles in patients with primary infection and suspected reinfection regarding their quantitative levels of IgG antibodies, IgG avidity, and neutralizing antibodies.

	Primary infection, Group 1 (n=187)	Suspected reinfection, Group 2 (n=30)	P value
IgG, IU/L	121 (64-249)	2881 (1359-4920)	<0.001
IgG avidity, %	15.9 (12.8-20.7)	80.5 (70.3-90.0)	<0.001
NT titers	35 (20-60)	480 (150-1280)	<0.001

Abbreviations: IU/L: international units per liter, NT: neutralization assay

Supplementary Table S2. Characterization of available reverse transcriptase polymerase chain reaction (RT-PCR) results in groups 1-3.

		PCR from serum	PCR from oral fluid/throat swap	PCR from urine
Primary infection, Group 1 (n=187)	Available (% of all)	187 (100%)	82 (43.9%)	55 (29.4%)
	Positive result	177	82	55
	Negative result	10	0	0
Suspected reinfection, Group 2 (n=30)	Available (% of all)	30 (100%)	14 (46.7%)	13 (43.3%)
	Positive result	24	14	13
	Negative result	6	0	0
Controls, Group 3 (n=153)	Available (% of all)	153 (100%)	24 (15.7%)	14 (9.2%)
	Positive result	0	0	0
	Negative result	153	24	14

Supplementary Table S3. Comparison of agreement between the IgM ELISAs **(A)** in the overall cohort (n=370), **(B)** in primary infection setting (n=236) and **(C)** in the setting of high Anti-MeV-IgG avidity (n=134), using Cohen's κ.

(A)

		Test B		
		-	~	+
Test A	-	138	9	34
	~	12	1	9
	+	10	0	157

		Test C		
		-	~	+
Test B	-	148	7	5
	~	5	1	4
	+	8	3	189

Cohen's κ=0.632, p<0.001

Cohen's κ=0.834, p<0.001

		Test C		
		-	~	+
Test A	-	140	8	33
	~	13	0	9
	+	8	3	156

		Test D		
		-	~	+
Test B	-	138	1	21
	~	8	1	1
	+	18	5	177

Cohen's κ=0.632, p<0.001

Cohen's κ=0.718, p<0.001

		Test D			76
		-	~	+	77
Test A	-	140	5	36	
	~	10	0	12	
	+	14	2	151	

		Test D		
		-	~	+
Test C	-	142	2	17
	~	4	1	6
	+	18	4	176

Cohen's κ=0.604, p<0.001

Cohen's κ=0.734, p<0.001

(B)

Test A \ Test B	-	~	+
-	54	6	22
~	5	0	8
+	4	0	137

Test B \ Test C	-	~	+
-	59	2	2
~	3	0	3
+	4	2	161

Cohen's κ=0.605, p<0.001

Cohen's κ=0.841, p<0.001

Test A \ Test C	-	~	+
-	59	2	21
~	5	0	8
+	2	2	137

Test B \ Test D	-	~	+
-	59	1	3
~	4	1	1
+	8	3	156

Cohen's κ=0.648, p<0.001

Cohen's κ=0.807, p<0.001

		Test D		96
		-	~	+ 97
Test A	-	60	4	18
	~	5	0	8
	+	6	1	134

		Test D		
		-	~	+
Test C	-	60	2	4
	~	1	1	2
	+	10	2	154

100

101 Cohen's κ=0.636, p<0.001

Cohen's κ=0.797, p<0.001

(C)

Test A \ Test B	-	~	+
-	84	3	12
~	7	1	1
+	6	0	20

Test B \ Test C	-	~	+
-	89	5	3
~	2	1	1
+	4	1	28

Cohen's κ=0.479, p<0.001

Cohen's κ=0.720, p<0.001

Test A \ Test C	-	~	+
-	81	6	12
~	8	0	1
+	6	1	19

Test B \ Test D	-	~	+
-	79	0	18
~	4	0	0
+	10	2	21

Cohen's κ=0.405, p<0.001

Cohen's κ=0.404, p<0.001

		Test D		116
		-	~	+ 117
Test A	-	80	1	18
	~	5	0	4
	+	8	1	17

		Test D		
		-	~	+
Test C	-	82	0	13
	~	3	0	4
	+	8	2	22

120

121 Cohen's κ=0.358, p<0.001

Cohen's κ=0.488, p<0.001

Supplementary Table S4. Sensitivity and specificity of IgM tests to diagnose acute MeV infections when excluding borderline results from calculations.

		Test A	Test B	Test C	Test D
Overall cohort	**Sensitivity**	72.6% (66.2-78.2%)	90.0% (85.1-93.3%)	86.4% (81.1-90.4%)	81.0% (75.1-85.7%)
	Specificity	88.6% (82.2-92.8%)	92.1% (86.6-95.4%)	90.4% (84.6-94.2%)	81.1% (74.1-86.5%)
Primary infection setting	**Sensitivity**	75.6% (68.8-81.3%)	89.6% (84.4-93.3%)	87.6% (82.0-91.6%)	85.3% (79.4-89.7%)
	Specificity	88.4% (75.5-94.9%)	93.6% (82.8-97.8%)	91.5% (80.1-96.6%)	91.7% (80.5-96.1%)
High Anti-MeV-IgG avidity setting	**Sensitivity**	53.6% (35.8-70.5%)	92.3% (75.9-98.6%)	78.6% (60.5-89.8%)	51.9% (34.0-69.3%)
	Specificity	88.7% (80.8-93.6%)	91.4% (84.4-95.4%)	89.9% (82.4-94.4%)	76.2% (67.2-83.3%)
Number of patients excluded		22	11	12	7

Supplementary Table S5. Diagnostic indices of IgM tests for diagnosing acute MeV infection if two tests are combined in the overall cohort, in the primary infection setting and in the setting of high Anti-MeV-IgG avidity. Diagnostic indices were calculated grading "borderline" results as "negative" and using the cut-offs according to the manufacturer. Two ways of combination were analyzed: **(A)** If any of the two tests reported a positive result, the sample was graded as positive. **(B)** If both of the two tests reported a positive result, the sample was graded as positive.

A

		Test A+B	Test A+C	Test A+D	Test B+C	Test B+D	Test C+D
Overall cohort (n=370)	**Sensitivity**	87.6% (82.5-91.3%)	85.8% (80.6-89.9%)	82.5% (76.9-87.0%)	88.5% (83.6-92.1%)	88.0% (83.0-91.7%)	87.1% (82.0-90.9%)
	Specificity	86.9% (80.7-91.4%)	85.0% (78.5-89.8%)	76.55 (69.2-82.5%)	88.9% (82.9-93.0%)	79.7% (72.7-85.3%)	79.1% (72.0-84.8%)
Primary infection setting (n=236)	**Sensitivity**	88.3% (82.9-92.1%)	87.2% (81.7-91.3%)	85.6% (79.9-89.9%)	89.4% (84.1-93.0%)	88.8% (83.5-92.6%)	88.8% (83.5-92.6%)
	Specificity	89.6% (77.8-95.5%)	87.5% (75.3-94.1%)	87.5% (75.3-94.1%)	91.7% (80.5-96.7%)	91.7% (80.5-96.7%)	89.6% (77.8-95.5%)
High Anti-MeV-IgG avidity setting (n=134)	**Sensitivity**	82.8% (65.5-92.4%)	75.9% (57.9-87.8%)	62.1% (44.0-77.3%)	82.8% (65.5-92.4%)	82.8% (65.5-92.4%)	75.9% (57.9-87.8%)
	Specificity	85.7% (77.8-91.2%)	83.8% (75.6-89.6%)	71.4% (62.2-79.2%)	87.6% (80.0-92.6%)	74.3% (65.2-81.7%)	74.3% (65.2-81.7%)

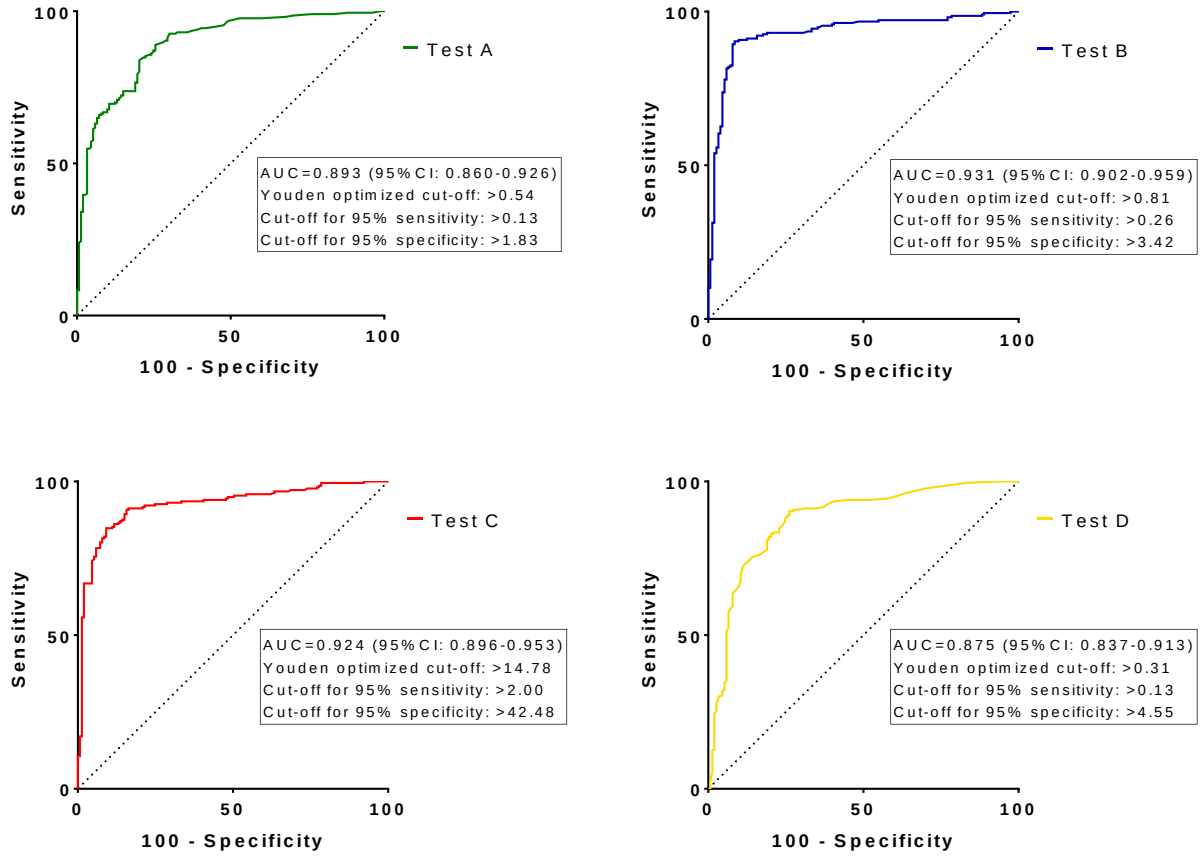
B

		Test A+B	Test A+C	Test A+D	Test B+C	Test B+D	Test C+D
Overall cohort (n=370)	**Sensitivity**	68.7% (62.2-74.5%)	68.7% (62.2-74.5%)	65.4% (58.9-71.5%)	83.0% (77.4-87.4%)	77.0% (80.9-82.1%)	76.0% (69.9-81.2%)
	Specificity	94.8% (90.0-97.3%)	95.4% (90.9-97.8%)	94.1% (89.2-96.9%)	94.1% (89.2-96.9%)	93.5% (88.4-96.4%)	92.8% (87.6-95.9%)
Primary infection setting (n=236)	**Sensitivity**	71.3% (64.4-77.3%)	71.3% (64.4-77.3%)	69.7% (62.8-75.8%)	84.0% (78.1-88.6%)	81.4% (75.2-86.3%)	80.3% (74.1-85.4%)
	Specificity	93.8% (83.2-97.9%)	93.8% (83.2-97.9%)	93.8% (83.2-97.9%)	93.8% (83.2-97.9%)	93.8% (83.2-97.9%)	93.8% (83.-97.9%)
High Anti-MeV-IgG avidity setting (n=134)	**Sensitivity**	51.7% (34.4-68.6%)	51.7% (34.4-68.6%)	37.9% (22.7-56.0%)	75.9% (57.9-87.8%)	48.3% (31.4-65.6%)	48.3% (31.4-65.6%)
	Specificity	95.2% (89.3-98.0%)	96.2% (90.6-98.5%)	94.3% (88.1-97.4%)	94.3% (88.1-97.4%)	93.3% (86.9-96.7%)	92.4% (85.7-96.1%)

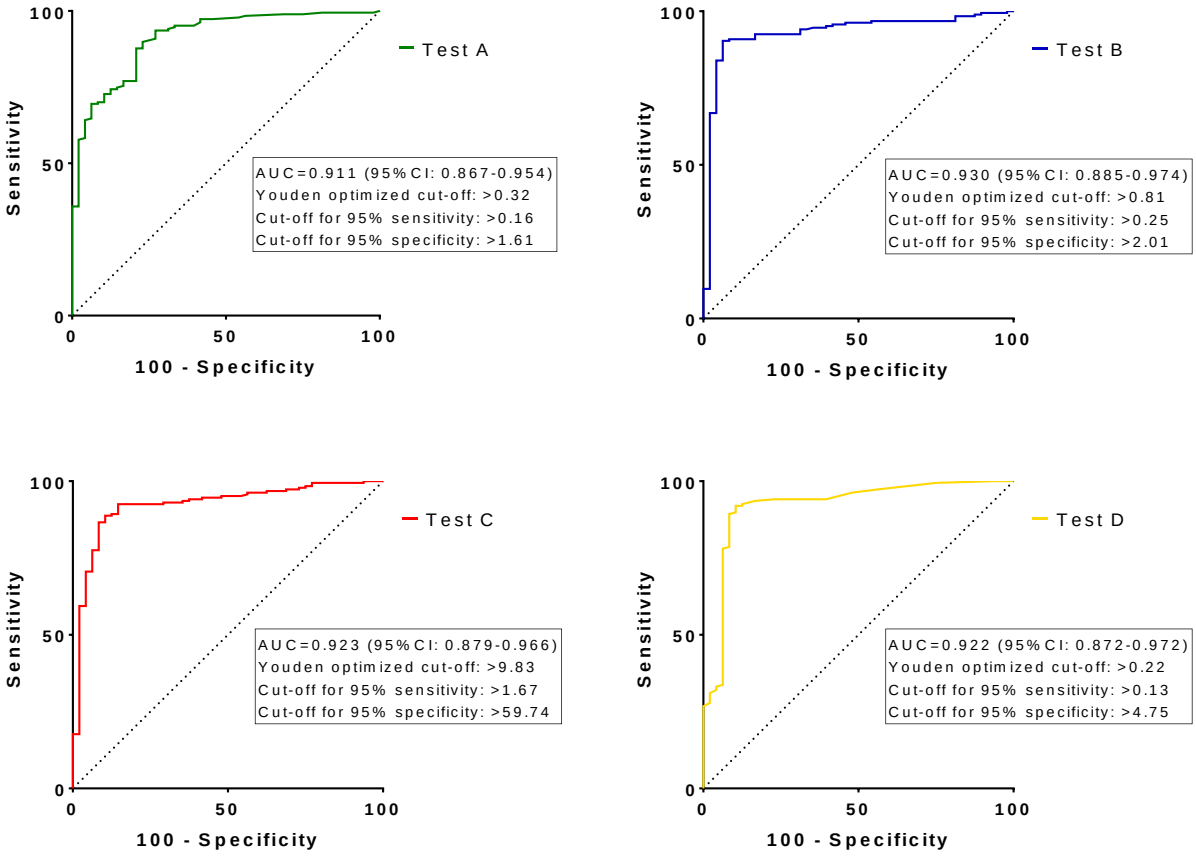
Supplementary Table S6. Results of IgM tests in patients with negative PCR from serum despite positive PCR from any other material. Borderline test results were graded as negative for calculation of sensitivity.

		Test A	Test B	Test C	Test D
RT-PCT negative samples (serum) in overall cohort (n=16)	**negative**	6	3	4	6
	borderline	0	1	0	0
	positive	10	12	12	10
	Sensitivity	62.5%	75.0%	75.0%	62.5%
RT-PCT negative samples (serum) In primary infection, Group 1 (n=10)	**negative**	3	2	3	3
	borderline	0	1	0	0
	positive	7	7	7	7
	Sensitivity	70.0%	70.0%	70.0%	70.0%
RT-PCT negative samples (serum) in suspected reinfection, Group 2 (n=6)	**negative**	3	1	1	3
	borderline	0	0	0	0
	positive	3	5	5	3
	Sensitivity	50.0%	83.5%	83.5%	50.0%

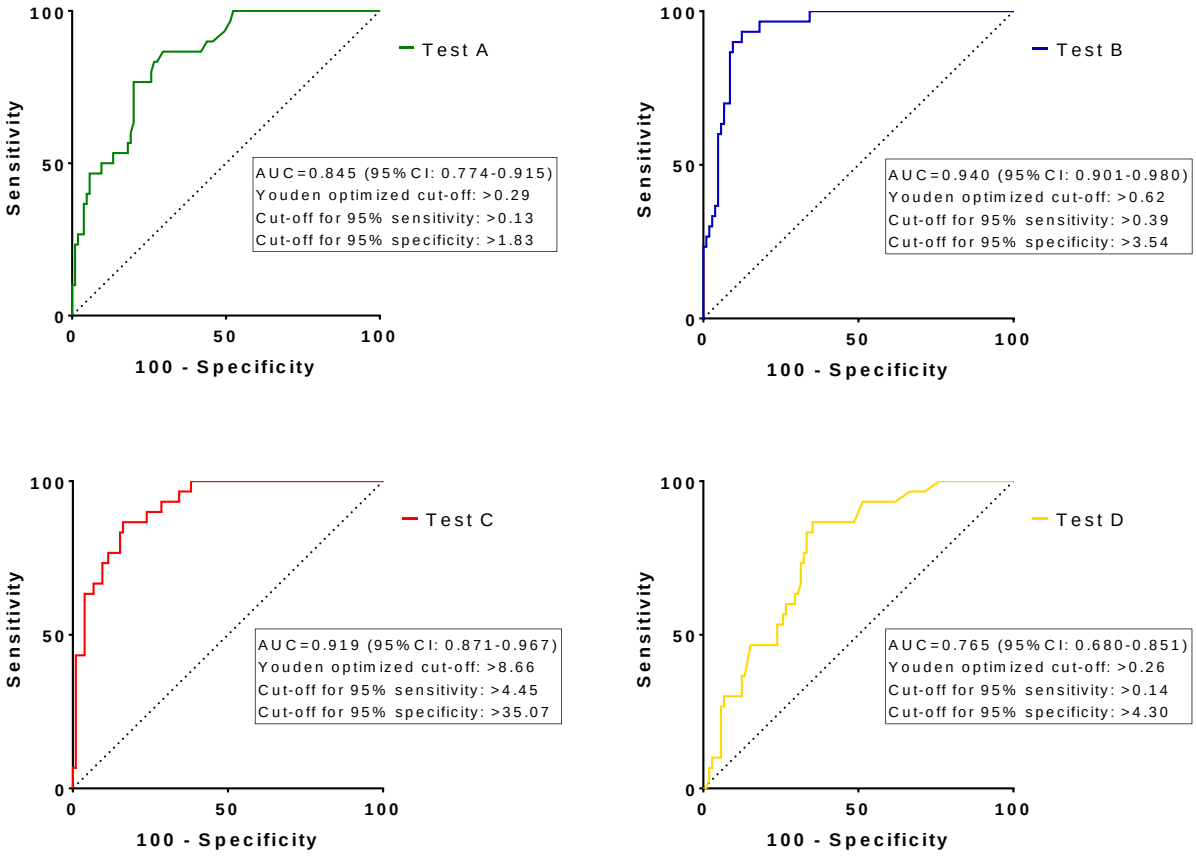
Supplementary Figure S1. Receiver operator characteristic (ROC) analysis of IgM tests to diagnose acute MeV infections in the overall cohort.



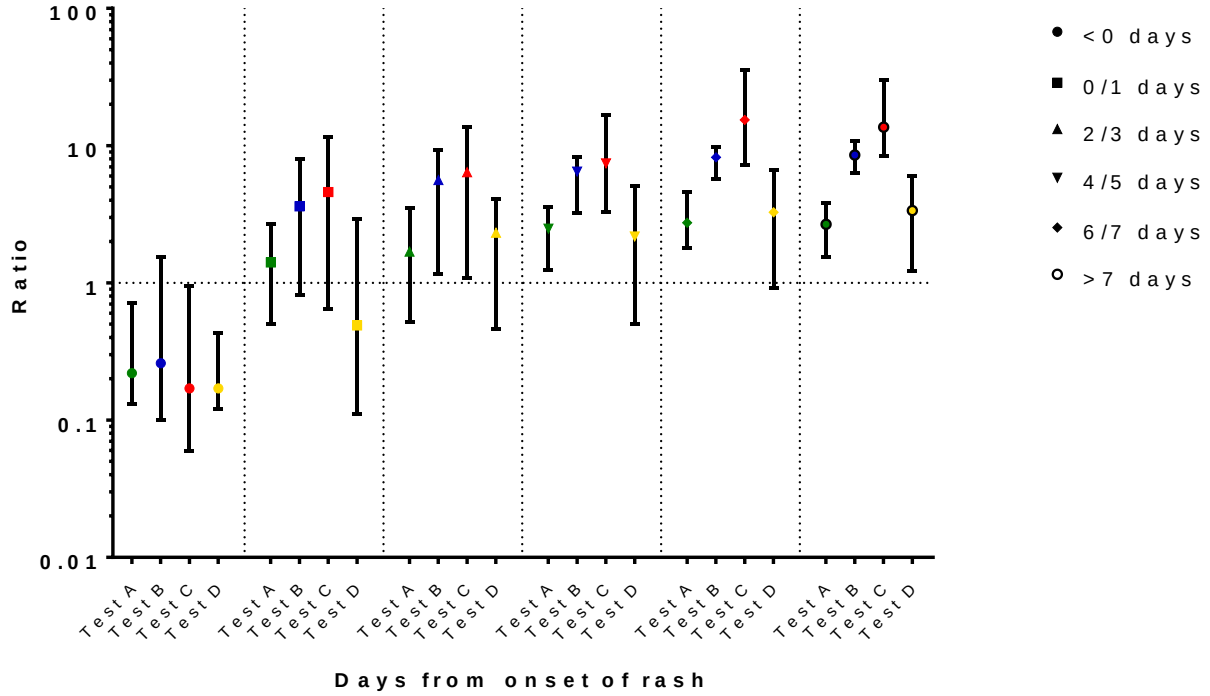
Supplementary Figure S2. Receiver operator characteristic (ROC) analysis of IgM tests to diagnose acute MeV infections in the primary infection setting.



Supplementary Figure S3. Receiver operator characteristic (ROC) analysis of IgM tests to diagnose acute MeV infections in the high Anti-MeV-IgG avidity setting.



Supplementary Figure S4. Comparison of the strength of antibody response measured with each test. Ratios were computed as IgM test values divided by the threshold when each test was considered positive for Test A-D. Median results and interquartile ranges of Test A-D at different days after onset of rash are displayed.



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