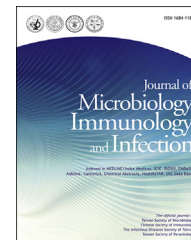


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Short Communication

Clinical application of rapid diagnostic test kit for SARS-CoV-2 antibodies into the field of patient care

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KEYWORDS

Rapid diagnostic test;
Antibody;
SARS-CoV-2;
Titration;
Point-of-care test

Abstract Clinical applicability of rapid diagnostic test kit for SARS-CoV-2 antibodies was evaluated. The kit detected antibodies from day 9–56 of illness. IgG bands were observed up to 1:1000 dilutions. The kit could detect 90.5% of IgG and 61.9% of IgM antibodies of mild febrile patients without pneumonia.

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Introduction

Global spread of coronavirus disease 2019 (COVID-19) outbreak is rapidly progressive.¹ Although detection of viral RNA by reverse transcription-polymerase chain reaction (RT-PCR) is the standard for the diagnosis of COVID-19,² need for serologic test is increasing for an alternative diagnostic method and/or seroprevalence studies. Rapid diagnostic test (RDT) kits for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies are easy to use and require 15 min for the test. To date, many RDT kits detecting SARS-CoV-2 IgM and/or IgG antibodies have been developed, but validation data with various clinical aspects are limited.^{3,4} For clinical application of RDT kits in the field of COVID-19 management, we evaluated performance according to the day of illness, type of specimens, feasibility as a point-of-care test (POCT), severity of illness, and with ten-fold titrations.

Methods

We performed SARS-CoV-2 IgM and IgG antibody tests using a RDT kit that had been used in recent report,⁴ in eight pneumonic COVID-19 patients and 21 mild febrile COVID-19 patients without pneumonia. SARS-CoV-2 infections of the evaluated patients were confirmed by RT-PCR. Pneumonic patients were admitted at tertiary care hospitals. Pneumonia was documented by chest X-ray and/or computed tomography. Onset of illness and clinical course were clearly documented. Day of illness was counted from the symptom onset (symptom onset day as D1), and the first week of illness denoted illness days from D1 to D7. Mild patients were cared at a life treatment center (LTC),⁵ meeting all of the following conditions: 1) mentally alert, 2) body-temperature below 37.5 °C at admission, 3) under 60 year of age, 4) no underlying disease, 5) non-smoker, and 6) no radiologic evidence of pneumonia. Among these mild patients, patients who experienced febrile sense, chilling, and/or myalgia were selected. Written informed consents were obtained from the patients. This study was approved by the Institutional Review Boards of the Samsung Medical Center (IRB No. 2020-03-113 and 2020-03-120).

The RDT kits using lateral flow immunoassay principle were assembled at Korea (Wells Bio Inc., Seoul, Korea) using the materials manufactured by Jiangsu Medomics Medical Technology (Nanjing, China).⁴ It is able to detect both IgM and IgG separately, targeting SARS-CoV-2 spike protein and supposed to use serum, plasma, and whole blood (WB) specimen and the manufacturer reported sensitivity of the kit was 88.66% and specificity was 90.63%. Test was performed according to the manufacturer's instructions.⁴ Interpretation of the test results is described in the [supplementary material](#) in detail.

Results

In overall, 52 specimens from 21 mild febrile and 8 pneumonic COVID-19 patients were evaluated ([Supplementary Table 1](#)). Among 43 specimens collected after the second week of illness (after D14), 41 specimens showed positive

IgG bands (95.3%) and 34 showed positive IgM bands (79.1%), including very weakly positive bands. Pictures of test results are presented in [Fig. 1](#) and [supplementary material](#) (large size of pictures as a PDF file).

Specimens from pneumonic COVID-19 patients and test for various clinical aspects

A total of 28 blood specimens from eight pneumonic COVID-19 patients were tested ([Fig. 1](#) and [Supplementary Figure 1 and 2](#)). Among 22 specimens collected from pneumonic patients after the 2nd week of illness (after D14), 22 specimens (100%) were positive for IgG bands and 21 (95.5%) were positive for IgM bands. IgG bands were clear and intense, while IgM bands were relatively fainter than IgG bands.

To assess the feasibility of RDT kit as a POCT, we tested three WB specimens at the bed-side of patients ([Fig. 1-\(a\)](#)). WB from arterial (taken from arterial line) and venous blood (taken from routine peripheral blood sampling) showed strong bands on IgG bands within two minutes after dropping two to three drops of blood on the test wells without adding additional dilution buffers, which were different from manufacturer's instruction as applying 15 µL WB and two to three drops of dilution buffer ([Supplementary figure 1](#); Exact titration of specimen amount could not be done for the bed-side tests, and dilution buffers were intentionally omitted to see if the kit can exhibit test results without adding buffer.).

For the evaluation of test performance according to the types of blood specimens, we compared WB, plasma, and serum specimens taken at the same day from patient 3 ([Supplementary Figure 2-\(a\)](#)). The intensity of IgG band was slightly stronger while IgM band was fainter with WB specimen, compared to plasma and serum specimens. Results using plasma and serum specimens did not show noticeable differences. This difference was also noted with the samples from Patient 4, taken by four-day interval ([Supplementary Figure 2-\(b\)](#)).

To see performance according to the day of illness, we tested serial serum specimens collected from a pneumonic patient (Patient 8) with moderate illness and a mild febrile patient (Patient 29, [Fig. 1-\(b\)](#)). For Patient 8, tests were negative by D5, and IgG and IgM bands were simultaneously positive on D9 with weak intensities for pneumonic patient. IgG bands became more intense with following specimens. As we did not collect specimen between D5 and D9, whether the kit could detect IgM earlier than IgG was not be evaluated for this pneumonic patient. Meanwhile, for Patient 29, the RDT kit could detect earlier response of IgM on D11. IgG band was noticed on D18 specimen for this mild febrile patient.

Diagnostic performance for mild febrile COVID-19 patients without pneumonia

To evaluate feasibility for seroprevalence study, convalescent sera from 21 mild febrile COVID-19 patients without pneumonia were evaluated ([Fig. 1-\(c\)](#)). Eight patients (38.1%) were male and the mean age was 32.2 years old. The sampling day ranged from D18 to D56. Nineteen

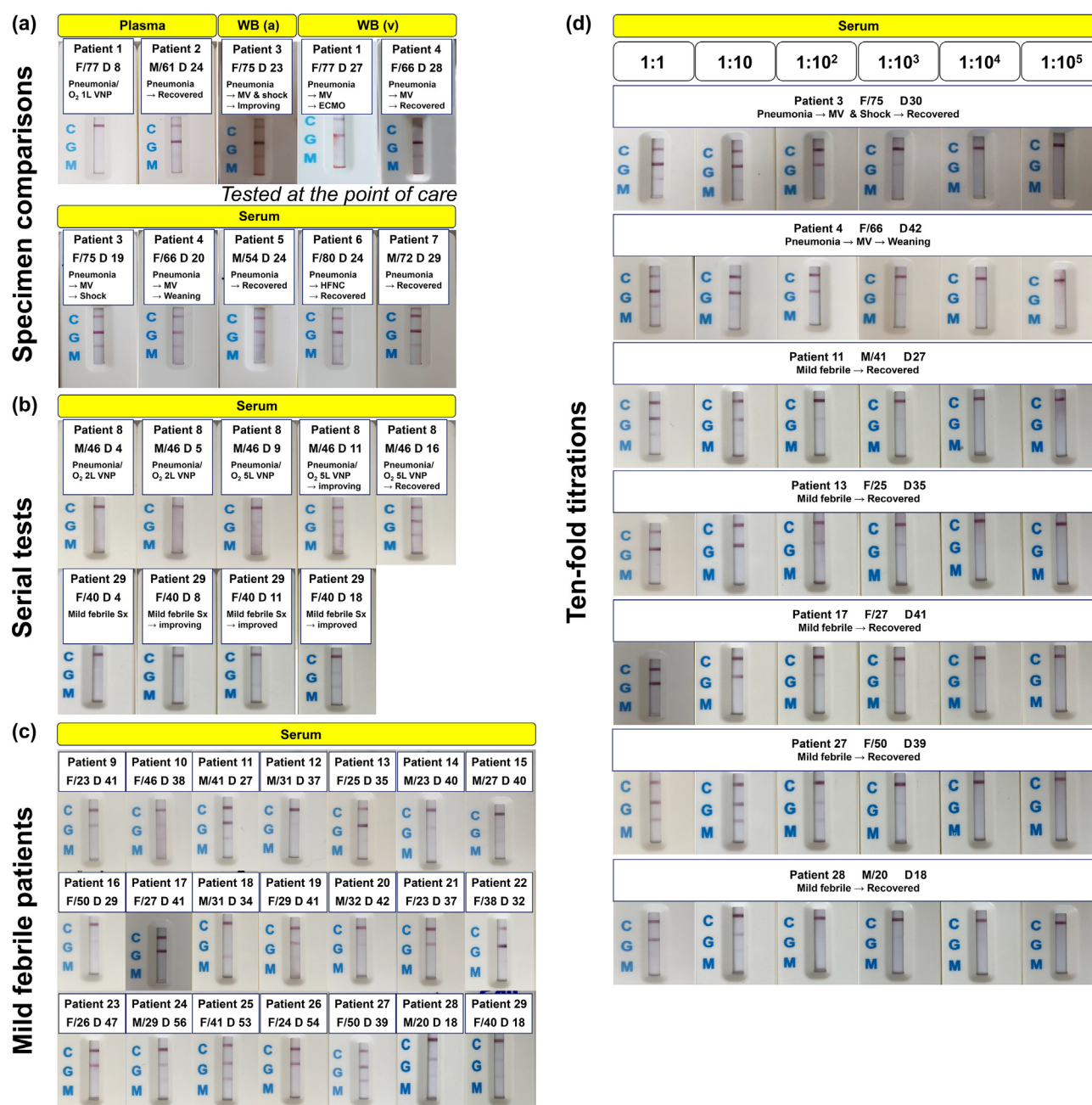


Figure 1. Tests for application of RDT kit for SARS-CoV-2 antibody into the field of COVID-19 patient management. (a) Tests using specimens from pneumonic COVID-19 patients. (b) Tests for serial specimens from a patient. (c) Tests using convalescent sera from mild febrile COVID-19 patients without pneumonia. (d) Titration tests with ten-fold dilutions of serum specimens. Pictures with larger size are presented as a supplementary material. Abbreviations: RDT, rapid diagnostic kit; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; WB, whole blood.

specimens (90.5%) were positive for IgG bands, including four weakly positive (Patient 10, 14, 20, and 29) and two very weakly positive bands (Patient 15 and 16). Thirteen specimens (61.9%) showed positive IgM bands, including three weakly positive (Patient 11 and 15) and seven very weakly positive bands (Patient 10, 13, 14, 19, 22, 24, and 28). One specimen was negative for both IgG and IgM, and overall intensity of bands was weaker than specimens from pneumonic patients.

Ten-fold titrations using convalescent sera of seven COVID-19 patients

Feasibility of RDT kit as a semi-quantitative method was also evaluated with ten-fold titrations. Convalescent sera collected from two pneumonic patients (Patient 3 and 4) and five mild febrile patients (Patient 11, 13, 17, 27, and 28) were diluted with PBS buffer from 1:10 to 1:100,000 (Fig. 1-(d)). Sera from two pneumonic patients showed

positive IgG intensity by 1:100 dilutions, and the bands became weakly positive at 1:1000 dilutions. Meanwhile, among five mild febrile patients, none of specimens showed positive IgG intensity at 1:100 dilutions (compared to pneumonic patients by the Chi-square test, $P = 0.048$); four showed weakly positive intensity and one was negative.

Discussion

Based on the initial performance data of serologic test kits for emerging diseases,³ clinical feasibility should be evaluated according to the purpose of serologic test. For the diagnostic purpose, we noticed the kit can detect IgG and IgM antibodies from D9 of illness. A published serologic study using enzyme linked immunosorbent assay (ELISA) suggested that the median time of seroconversion was D12 for IgM and D14 for IgG.⁶ Another report also presented presence of IgM and IgG antibodies from the 3rd week of illness.⁷ In the present test, it was noticed that the RDT kit detected IgG antibodies in 95.3% of specimens collected after the second week of illness (after D14), suggesting that the performance of the kit would be comparable to previous reports.

Of note, we also tested feasibility of RDT kit as a semi-quantitative method with ten-fold dilution of serum specimen, and noticed that RDT kit could detect IgG antibodies of 1:1000 diluted sera. As of the current COVID-19 pandemic, convalescent plasma infusion therapy is one of potential treatment options for severe COVID-19 patients.^{8,9} For the selection of donor plasma, measurement of functional anti-SARS-CoV-2 antibodies by neutralization test is necessary. However, facilities for neutralization tests are not readily available, non-culture based methods can be used for titrations of antibodies.¹⁰ Our finding provided that RDT kit can be used for a semi-quantitative method for assessing amount antibodies, which would be useful in resource limited settings.

Lastly, performance of detecting antibodies in convalescent serum of mild illness was evaluated. The main purpose of seroprevalence study in the current pandemic would be to detect potential undiagnosed COVID-19 patients, who are likely to be mild cases. As antibody response in mild illness would be weak, whether an antibody test kit can detect serologic response in these patients is an important issue. It was noticed that the kit can detect IgG antibodies with high detection rate in mild febrile patients without pneumonia. However, since bands in these patients were relatively faint and serologic response in non-febrile patients can be weaker, more sensitive method to detect weak serologic response should be further studied.

In conclusion, in the test for RDT kit for SARS-CoV-2 antibodies, it detected IgG response from D9 with 95.3% detection rate after the second week of illness (after D14). It could be used as POCT, a semi-quantitative method, and for seroprevalence studies.

Funding

None received.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmii.2020.07.003>.

Supplementary Materials

Interpretation of the test results

The test results of RDT kits were interpreted as the following categories: 1) positive, if the IgG or IgM band showed similar intensity with the control band; 2) weakly positive, if the IgG or IgM band are clearly visible, but much fainter than the control band; 3) very weakly positive, if the IgG or IgM band are visible with very faint intensity; and 4) negative, if the IgG or IgM band are invisible, while the control band is visible. Test results were interpreted at the time of test, and finally confirmed by the two investigators' agreement (J.-H.K. and K.R.P) based on the pictures. Although some of very weakly positive results are nearly invisible on the pictures due to relatively low resolution, they were more clear and visible at the time of test.

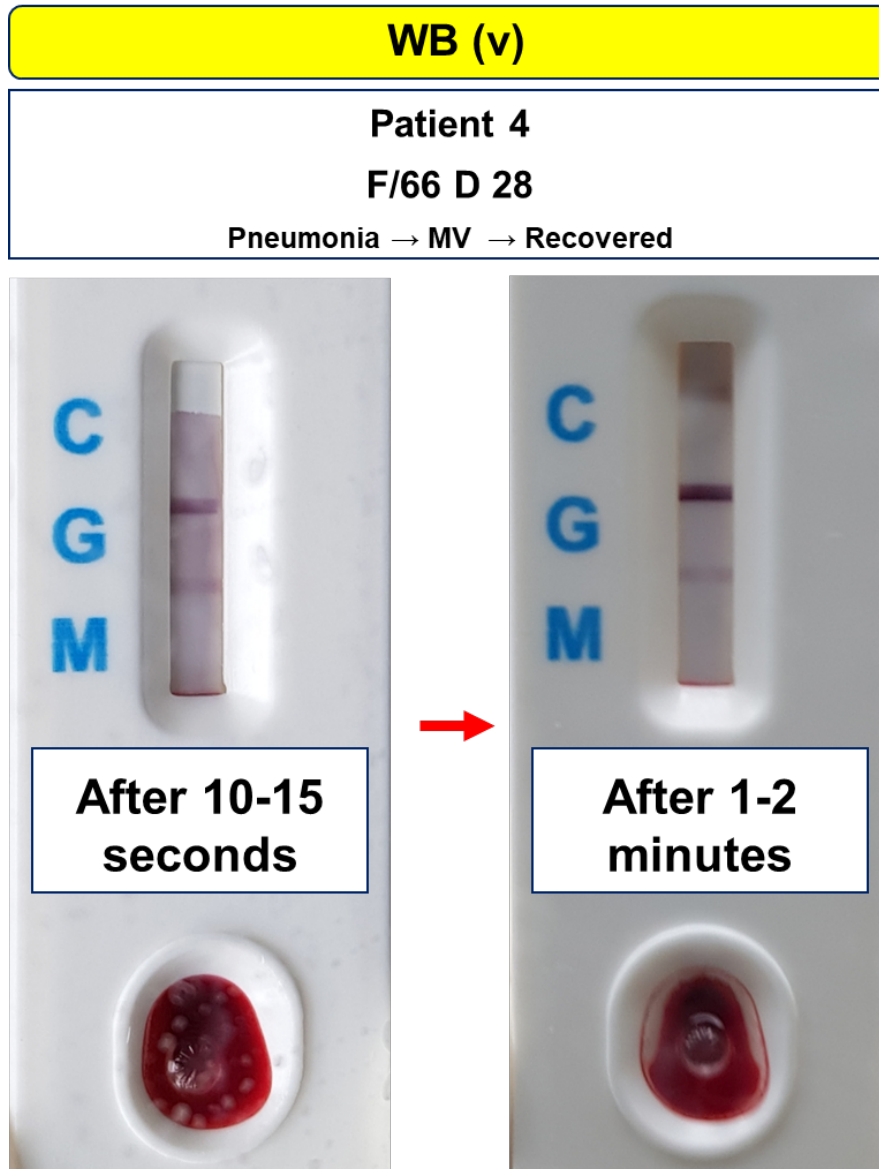
Supplementary Table 1. Summary of overall test result of the RDT kit for SARS-CoV-2 IgG and IgM antibodies

Categor y	Specimen number	Patient number	Se x	Age	Day	IgG	IgM	Picture location
<i>Pneu monic COVI D-19 patien ts</i>	1	1	F	77	8	neg.	neg.	Figure 1
	2	1	F	77	27	pos.	neg.	Figure 1
	3	2	M	61	24	pos.	very weak.	Figure 1
	4	3	F	75	19	pos.	pos.	Figure 1
	5	3	F	75	23	pos.	pos.	Figure 1
	6	3	F	75	27	pos.	pos.	Suppl. Figure 2
	7	3	F	75	30	pos.	pos.	Figure 1
	8	3	F	75	37	pos.	weak pos.	Picture not shown
	9	3	F	75	54	pos.	weak pos.	Picture not shown
	10	4	F	66	20	pos.	pos.	Figure 1
	11	4	F	66	21	pos.	pos.	Picture not shown
	12	4	F	66	28	pos.	pos.	Figure 1
	13	4	F	66	32	pos.	pos.	Picture not shown
	14	4	F	66	42	pos.	pos.	Figure 1
	15	4	F	66	53	pos.	pos.	Picture not shown
	16	4	F	66	56	pos.	pos.	Picture not shown
	17	5	M	54	22	pos.	pos.	Figure 1
	18	5	M	54	24	pos.	pos.	Picture not shown
	19	6	F	80	24	pos.	pos.	Figure 1
	20	6	F	80	25	pos.	pos.	Picture not shown
	21	7	M	72	29	pos.	pos.	Figure 1
	22	8	M	46	4	neg.	neg.	Figure 1
	23	8	M	46	5	neg.	neg.	Figure 1
	24	8	M	46	9	weak pos.	weak pos.	Figure 1
	25	8	M	46	11	pos.	pos.	Figure 1
	26	8	M	46	13	pos.	pos.	Picture not shown
	27	8	M	46	15	pos.	pos.	Picture not shown
	28	8	M	46	16	pos.	pos.	Figure 1
<i>Mild febril e COVI D-19 patien ts witho ut</i>	29	9	F	23	41	pos.	neg.	Figure 1
	30	10	F	46	38	weak pos.	very weak.	Figure 1
	31	11	M	41	27	pos.	weak pos.	Figure 1
	32	12	M	31	37	neg.	neg.	Figure 1
	33	13	F	25	35	pos.	very weak.	Figure 1
	34	14	M	23	40	weak pos.	very weak.	Figure 1
	35	15	M	27	40	very weak.	weak pos.	Figure 1
	36	16	F	50	29	very weak.	neg.	Figure 1

<i>pneu moni a</i>	37	17	F	27	41	pos.	neg.	Figure 1
	38	18	M	31	34	neg.	pos.	Figure 1
	39	19	F	29	41	pos.	very weak.	Figure 1
	40	20	M	32	42	weak pos.	neg.	Figure 1
	41	21	F	23	37	pos.	neg.	Figure 1
	42	22	F	38	32	pos.	very weak.	Figure 1
	43	23	F	26	47	pos.	neg.	Figure 1
	44	24	M	29	56	pos.	very weak.	Figure 1
	45	25	F	41	53	pos.	pos.	Figure 1
	46	26	F	24	54	pos.	neg.	Figure 1
	47	27	F	50	39	pos.	pos.	Figure 1
	48	28	M	20	18	pos.	very weak.	Figure 1
	49	29	F	40	4	neg.	neg.	Figure 1
	50	29	F	40	8	neg.	neg.	Figure 1
	51	29	F	40	11	neg.	weak pos.	Figure 1
	52	29	F	40	18	weak pos.	pos.	Figure 1

The test results of RDT kits were interpreted as the following categories: 1) positive: the IgG or IgM band showed similar intensity with the control band, 2) weakly positive: the IgG or IgM band are clearly visible, but much fainter than the control band, 3) very weakly positive: the IgG or IgM band are visible with very faint intensity, 4) negative: the IgG or IgM band are invisible, while the control band is visible.

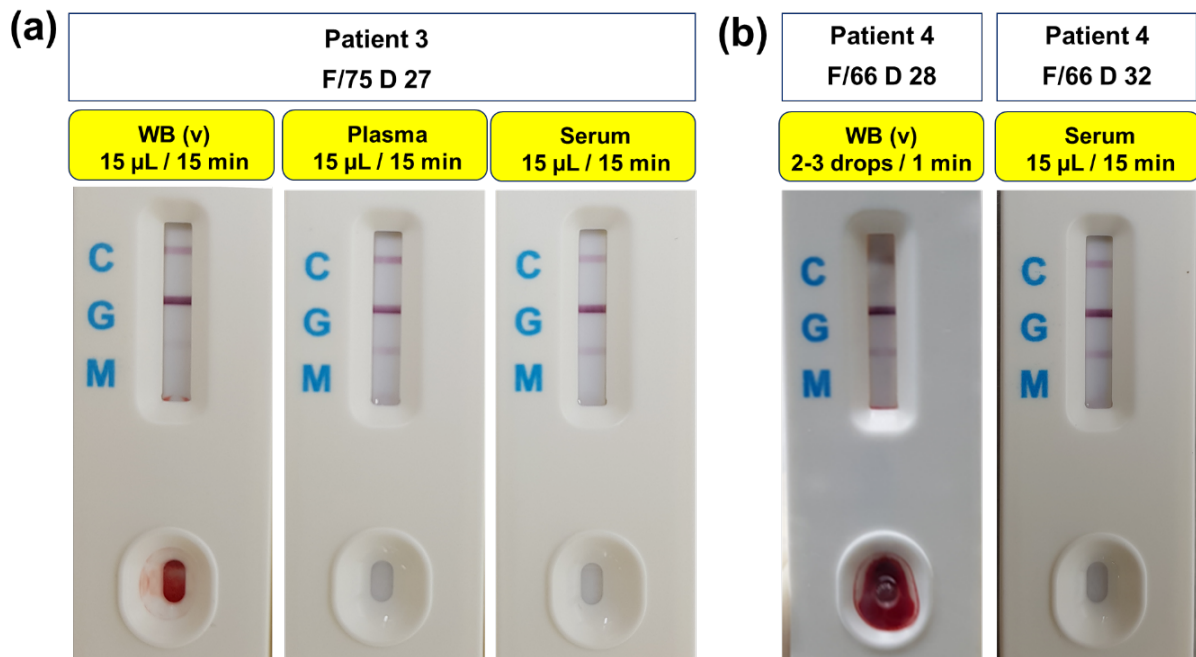
Abbreviations: RDT, rapid diagnostic kit; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; pos., positive; neg., negative; weak pos., weakly positive; very weak, very weakly positive



Supplementary Figure 1. Test of a RDT kit for SARS-CoV-2 antibody using venous whole blood at the patient's bed-side.

The kit rapidly showed clear IgG band 10 to 15 seconds after direct inoculation of two to three drops of whole blood without adding buffer. After 1 to 2 minutes, IgG band became more intense and the control band was visible. Exact titration of specimen amount could not be done for the bed-side tests, and dilution buffers were intentionally omitted to see if the kit can exhibit test results without adding buffer. Other tests expect the bed-side tests were performed according to the instruction.

Abbreviations: RDT, rapid diagnostic kit; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; WB, whole blood



Supplementary Figure 2. Test for a RDT kit for SARS-CoV-2 antibody comparing different types of blood specimens

(a) Venous WB, plasma, and serum specimens taken at the same day from Patient 3 were tested. IgG band was slightly more intense while IgM band was faint with the WB specimen, compared to the plasma and serum specimens. Tests by plasma and serum specimens did not show meaningful differences. (b) This difference was also noted with the samples from Patient 4, taken by four-days interval.

Abbreviations: RDT, rapid diagnostic kit; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; WB, whole blood

(a) Specimen comparisons

Plasma

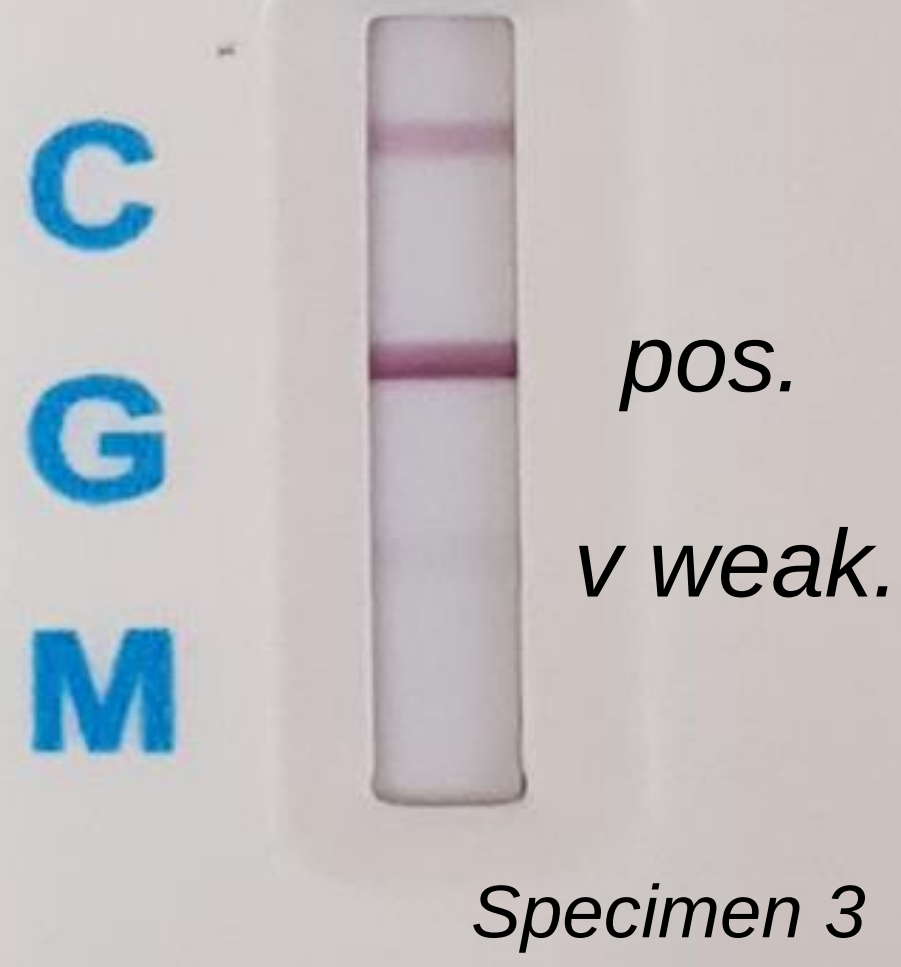
WB (a)

WB (v)

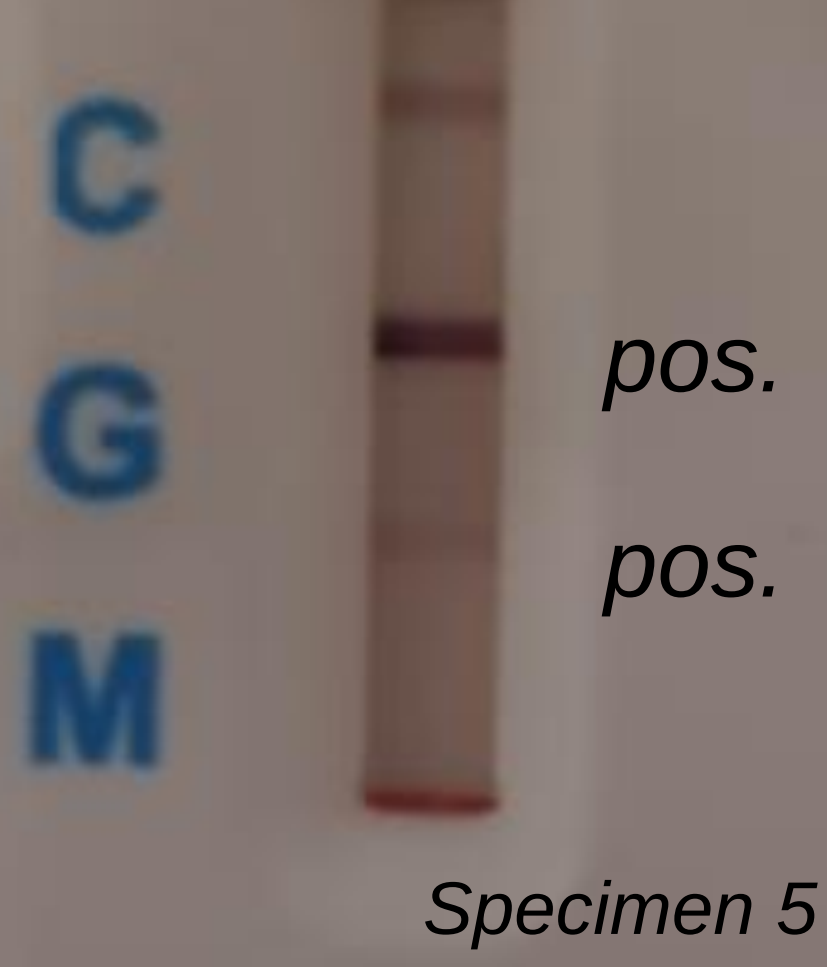
Patient 1 D8



Patient 2 D24



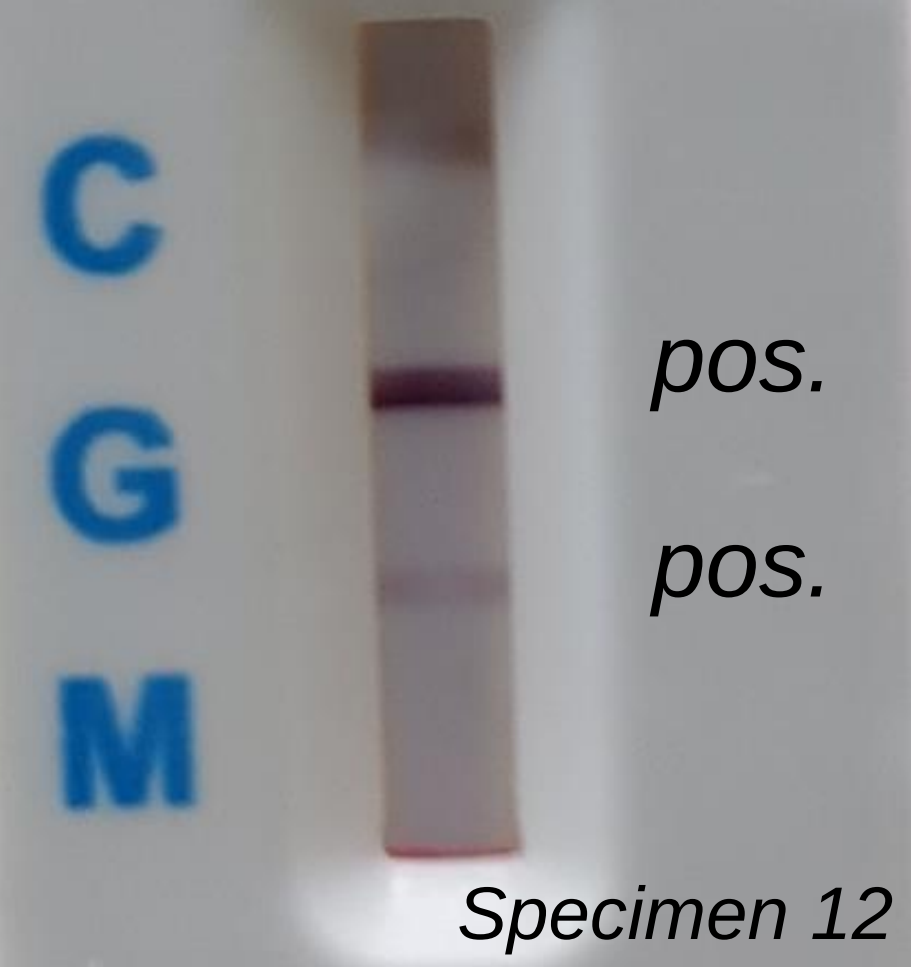
Patient 3 D23



Patient 1 D27



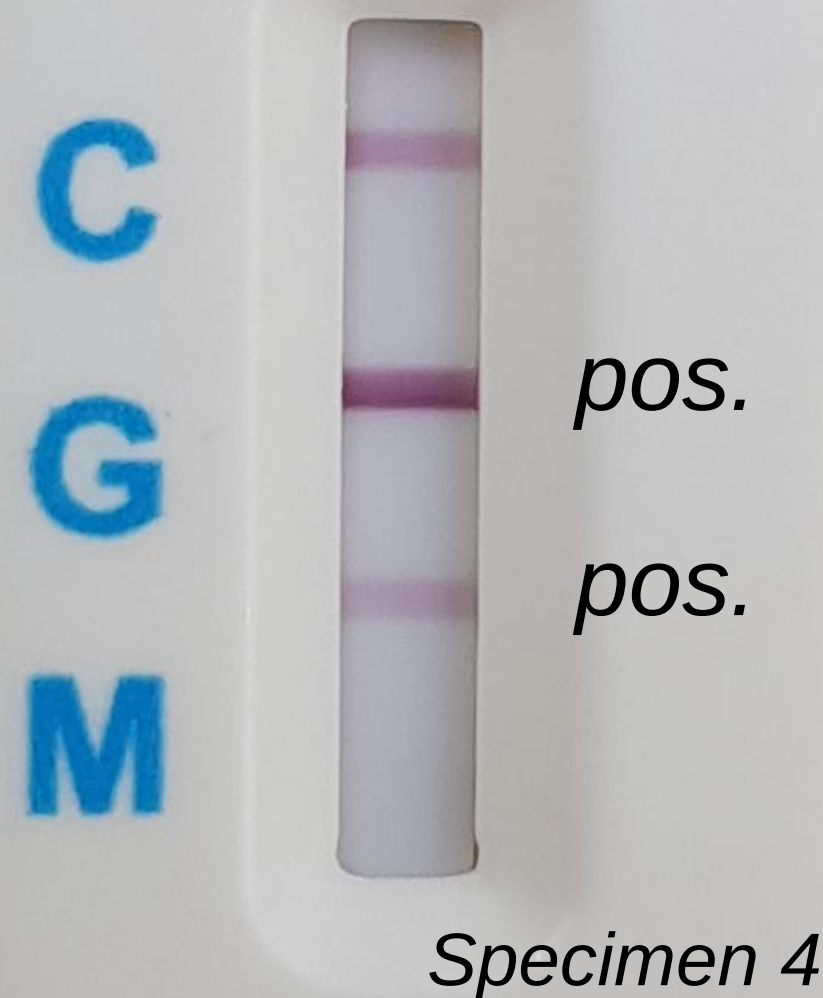
Patient 4 D28



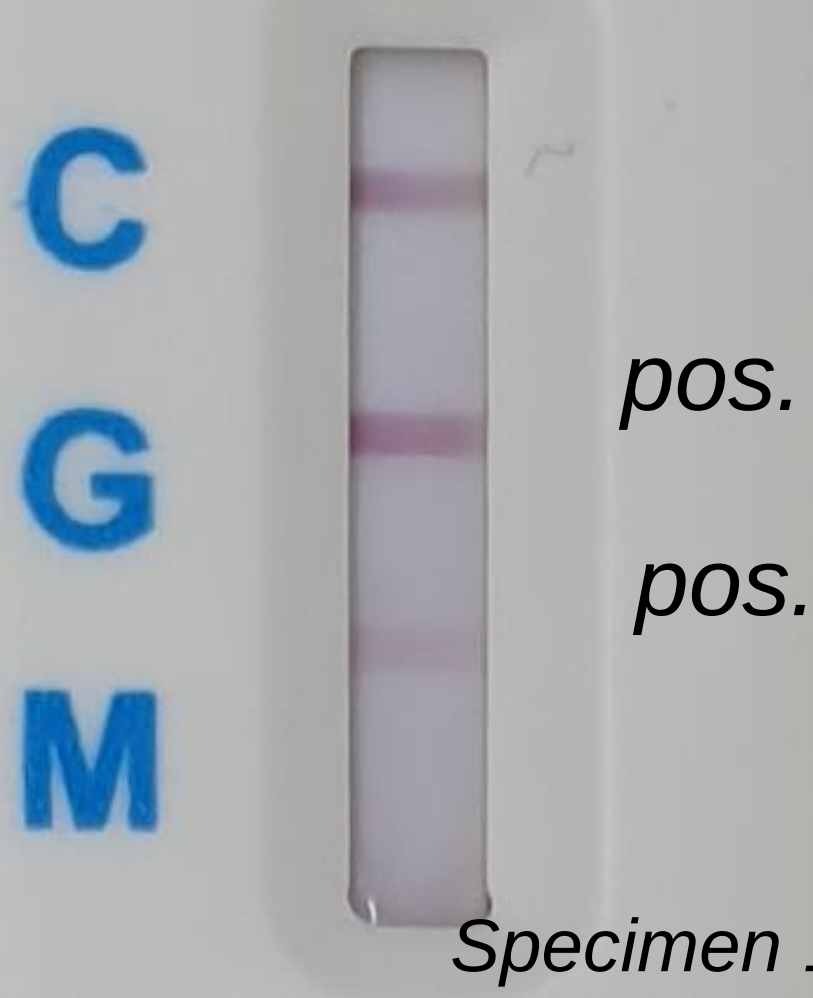
Tested at the point of care

Serum

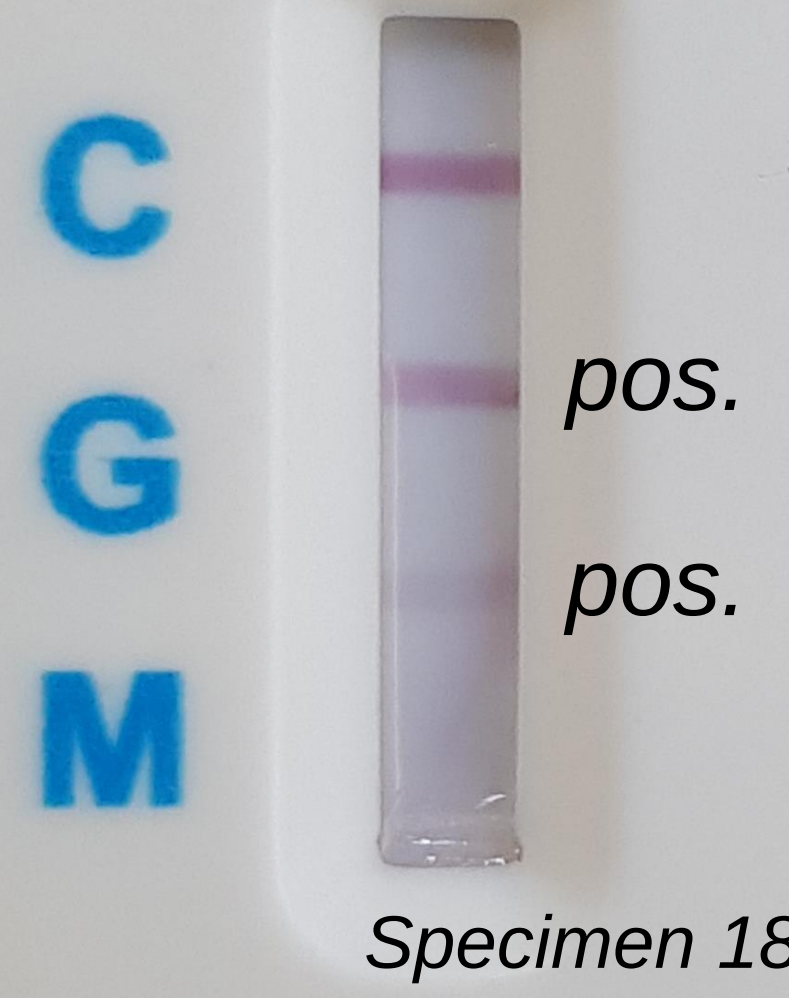
Patient 3 D19



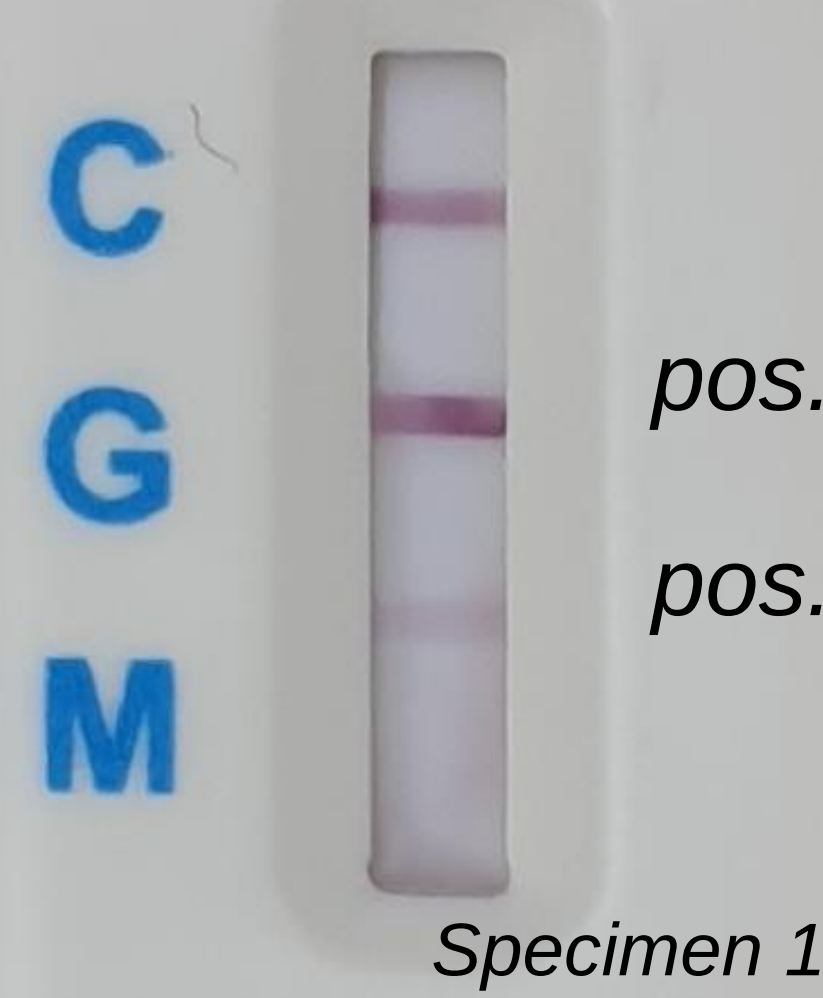
Patient 4 D20



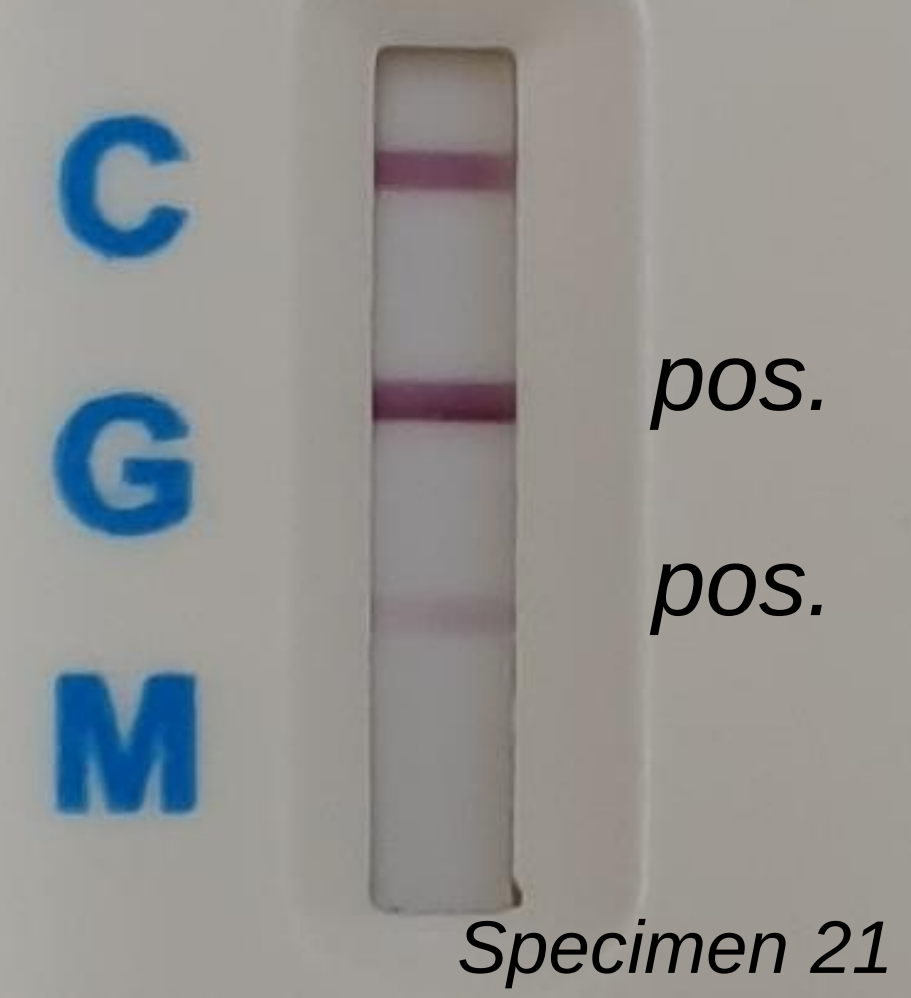
Patient 5 D24



Patient 6 D24



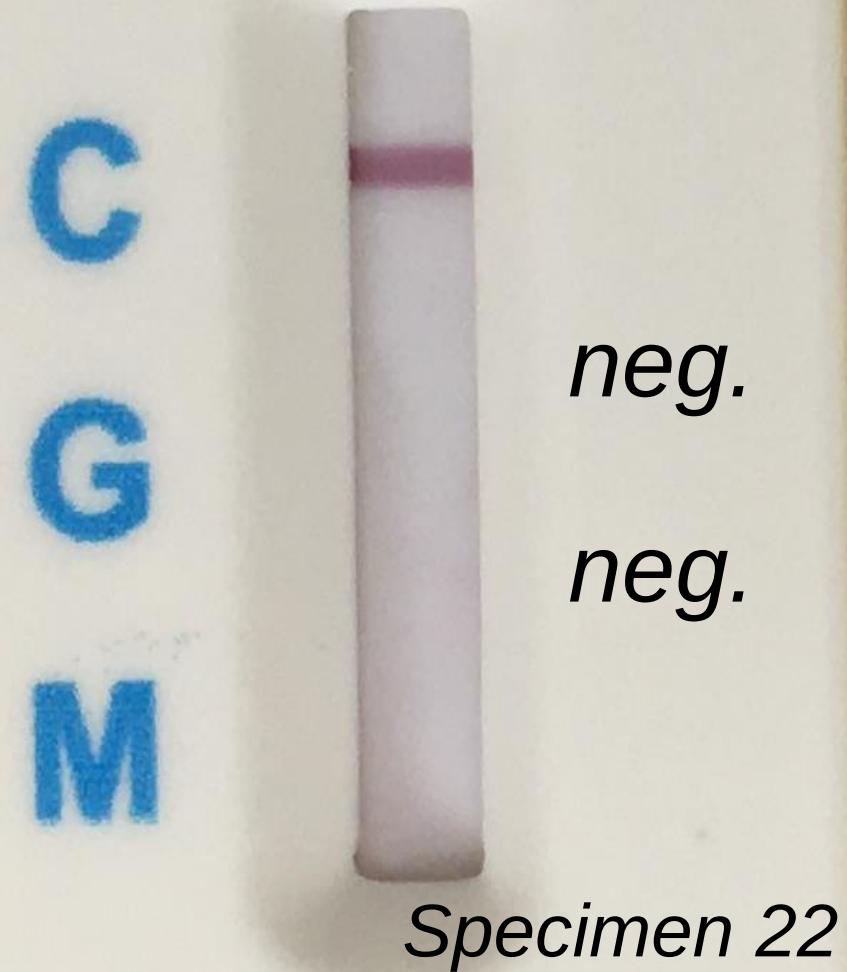
Patient 7 D29



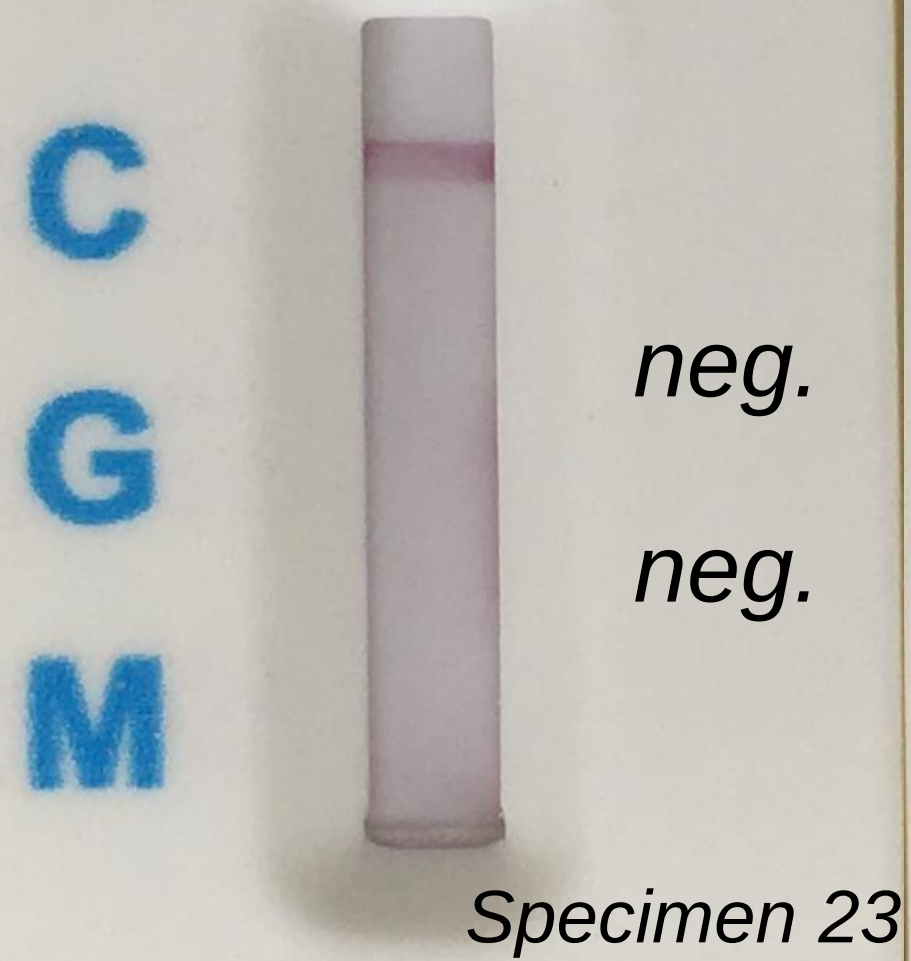
(b) Serial tests

Serum

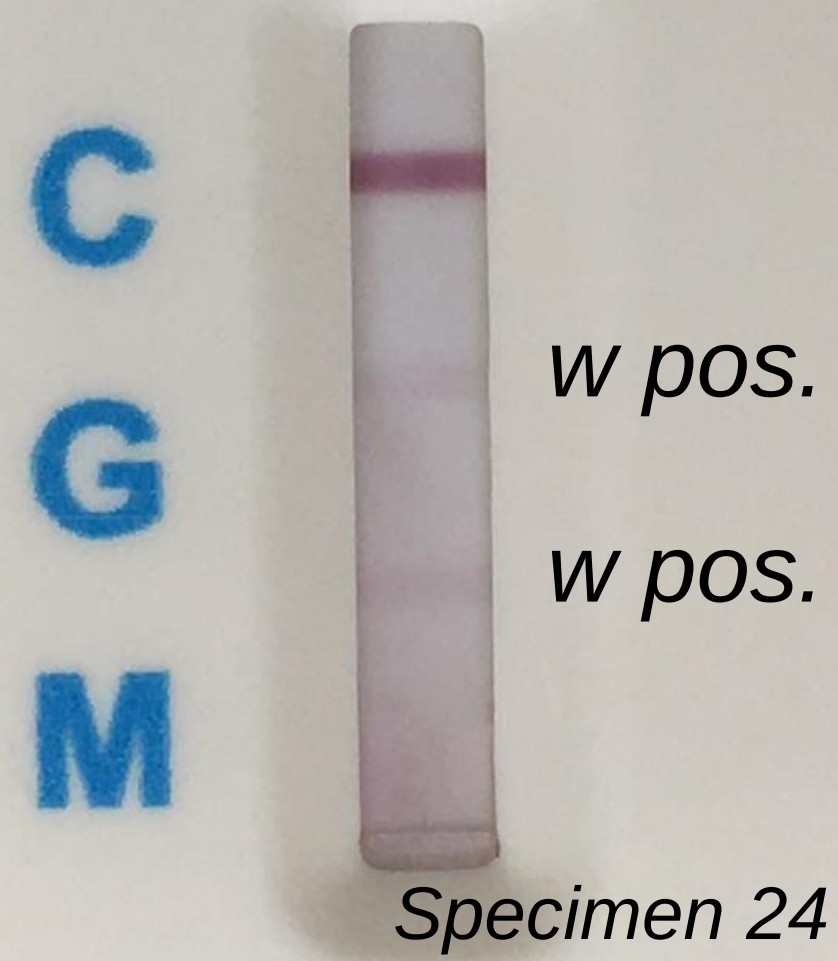
Patient 8 D4



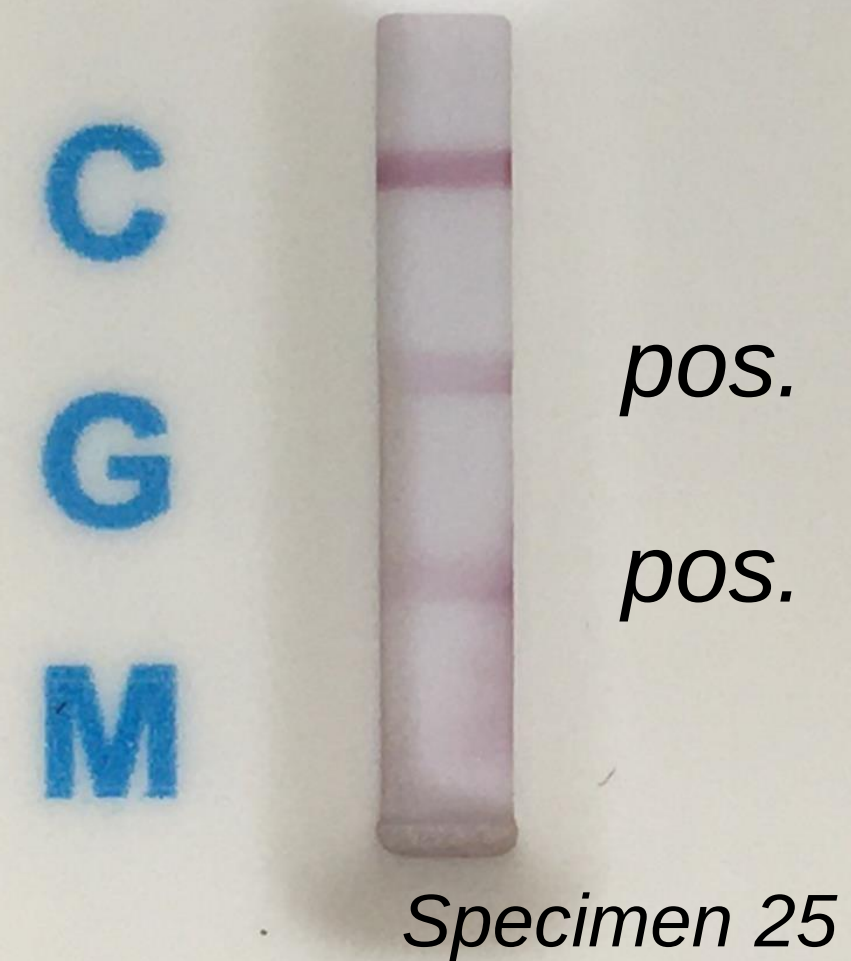
Patient 8 D5



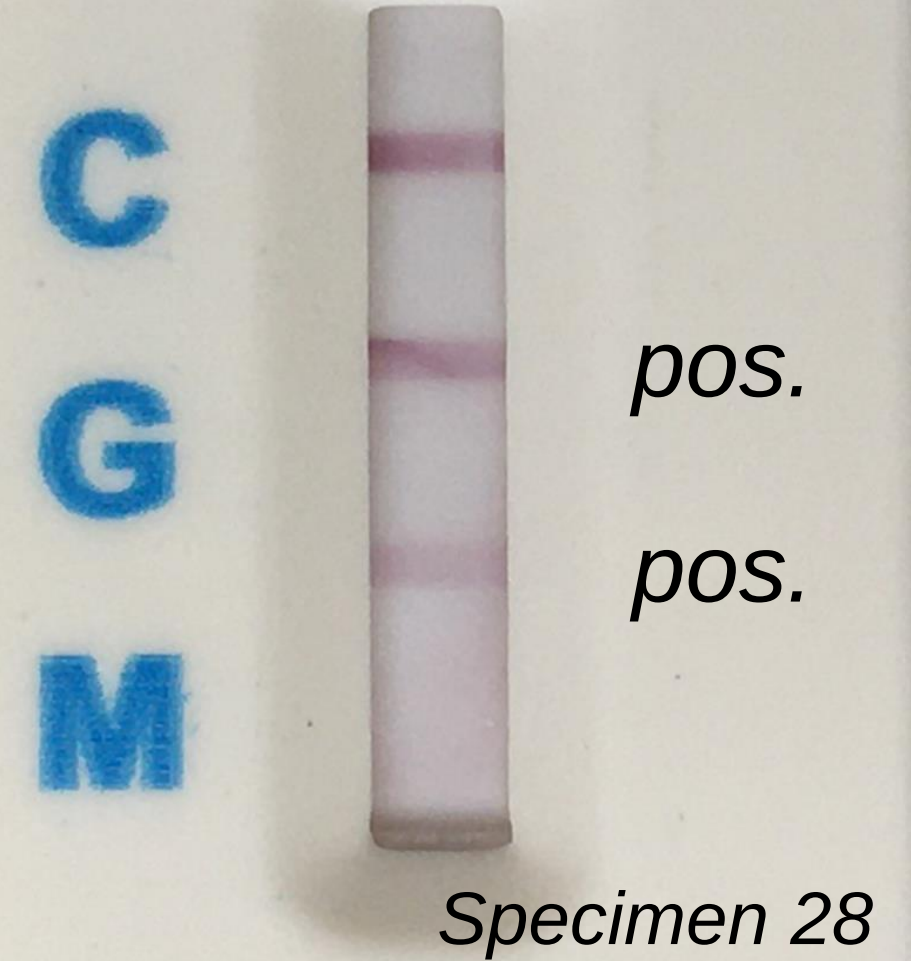
Patient 8 D9



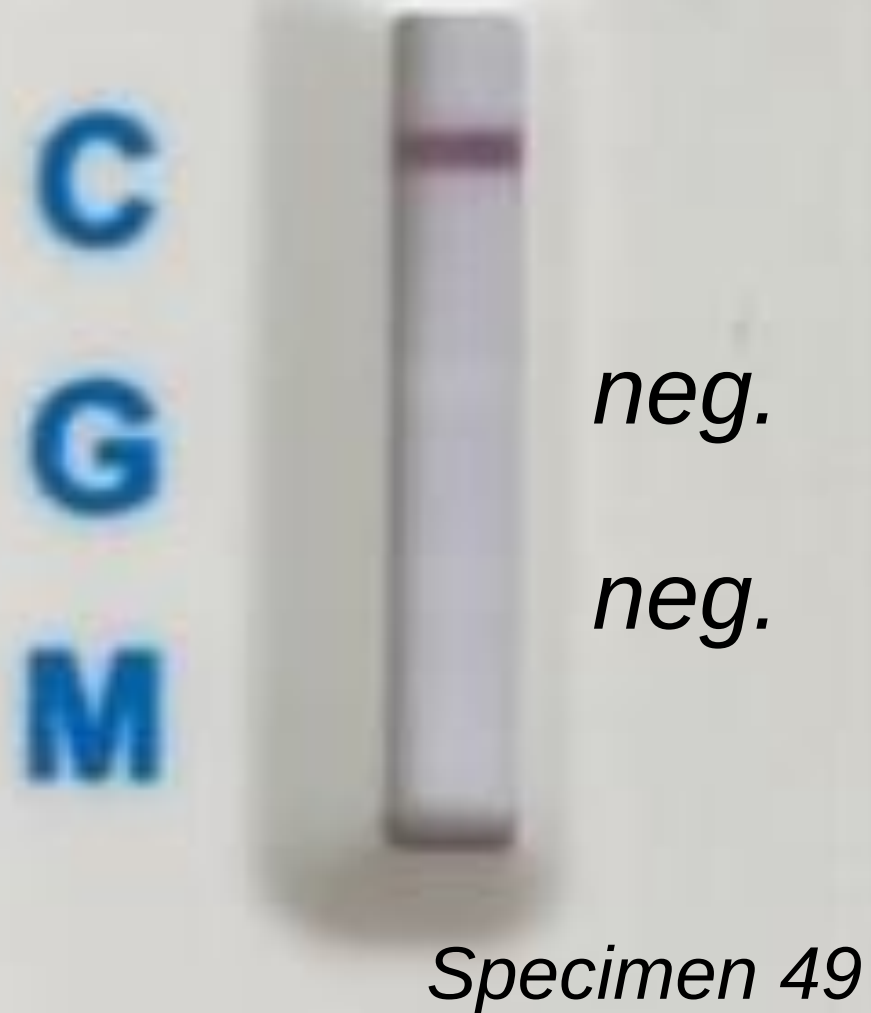
Patient 8 D11



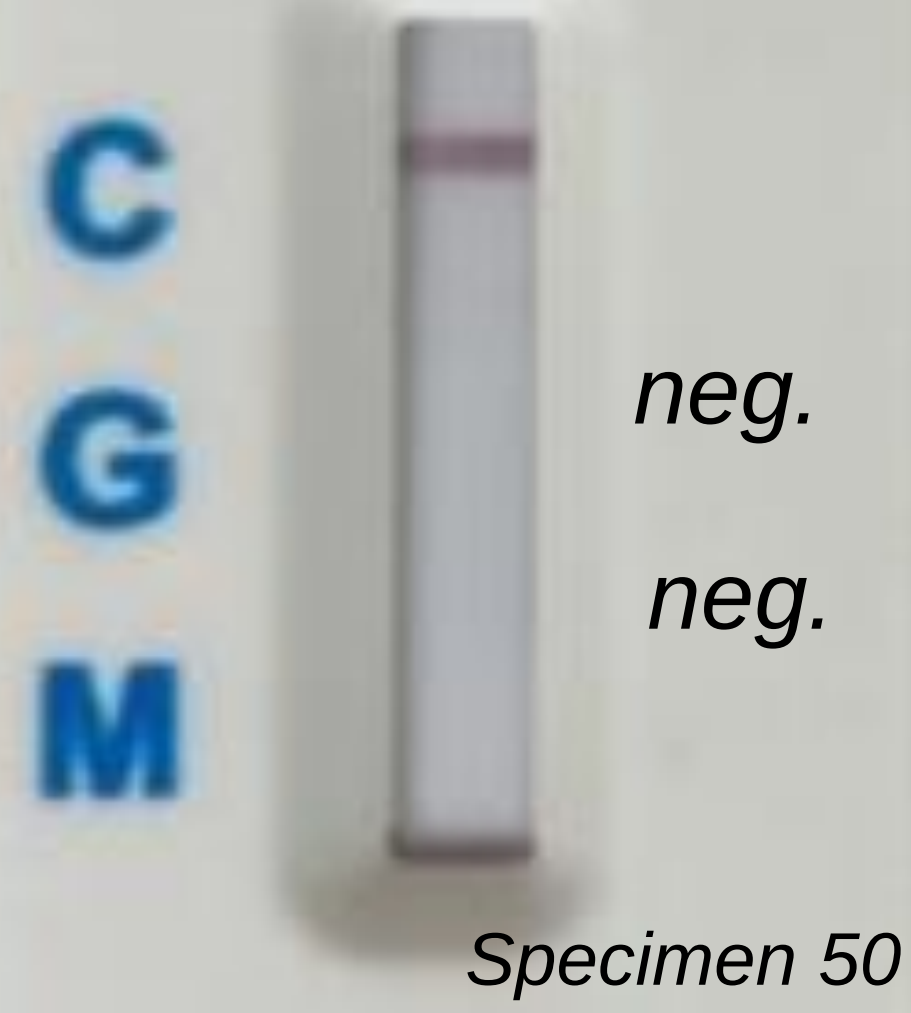
Patient 8 D16



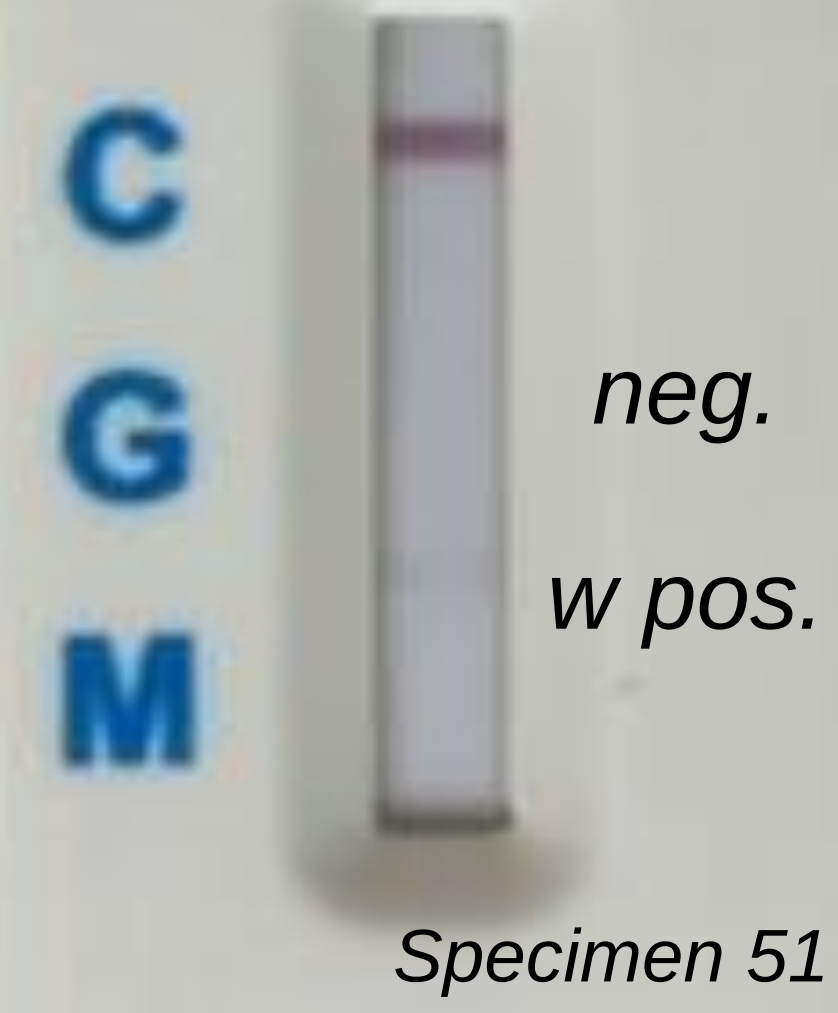
Patient 29 D4



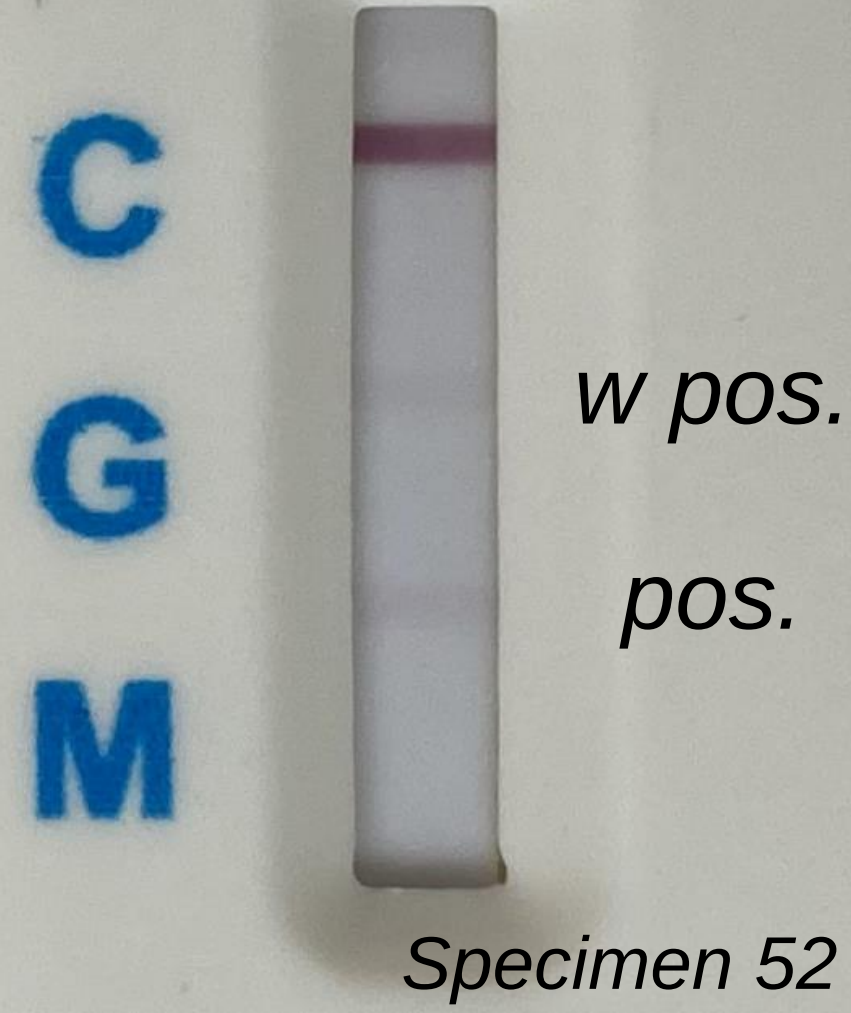
Patient 29 D8



Patient 29 D11



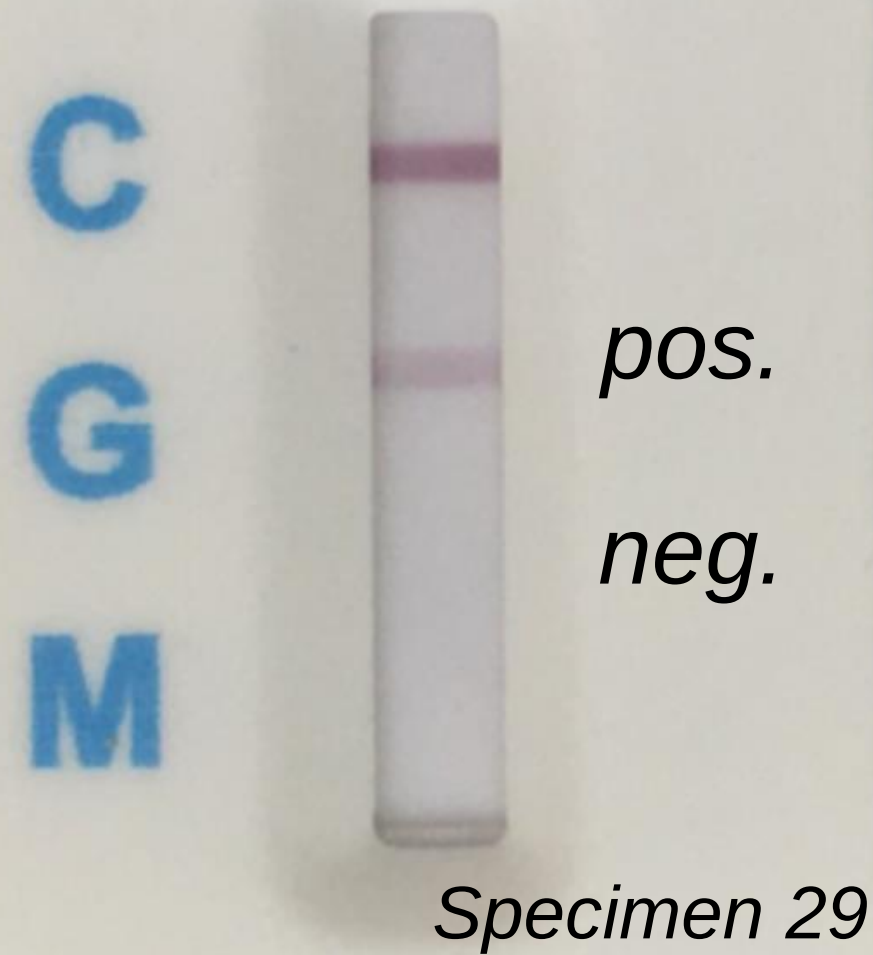
Patient 29 D18



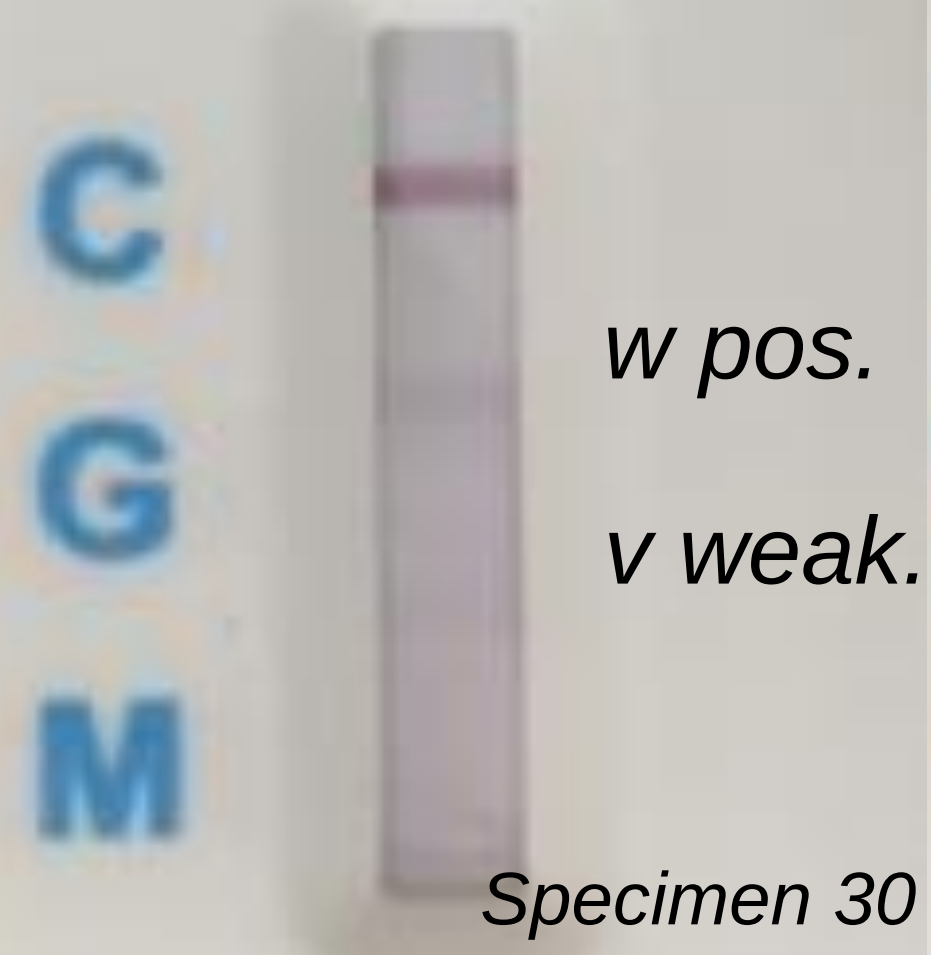
(c) Mild febrile patients (patient 9 – 18)

Serum

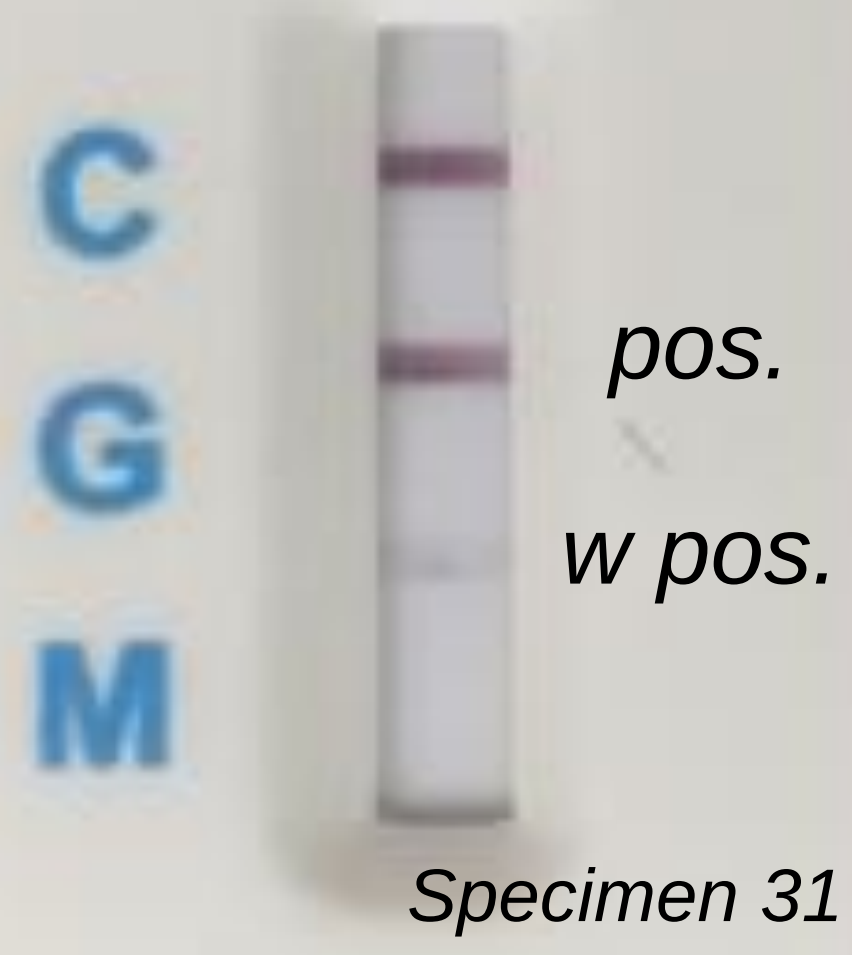
Patient 9 D41



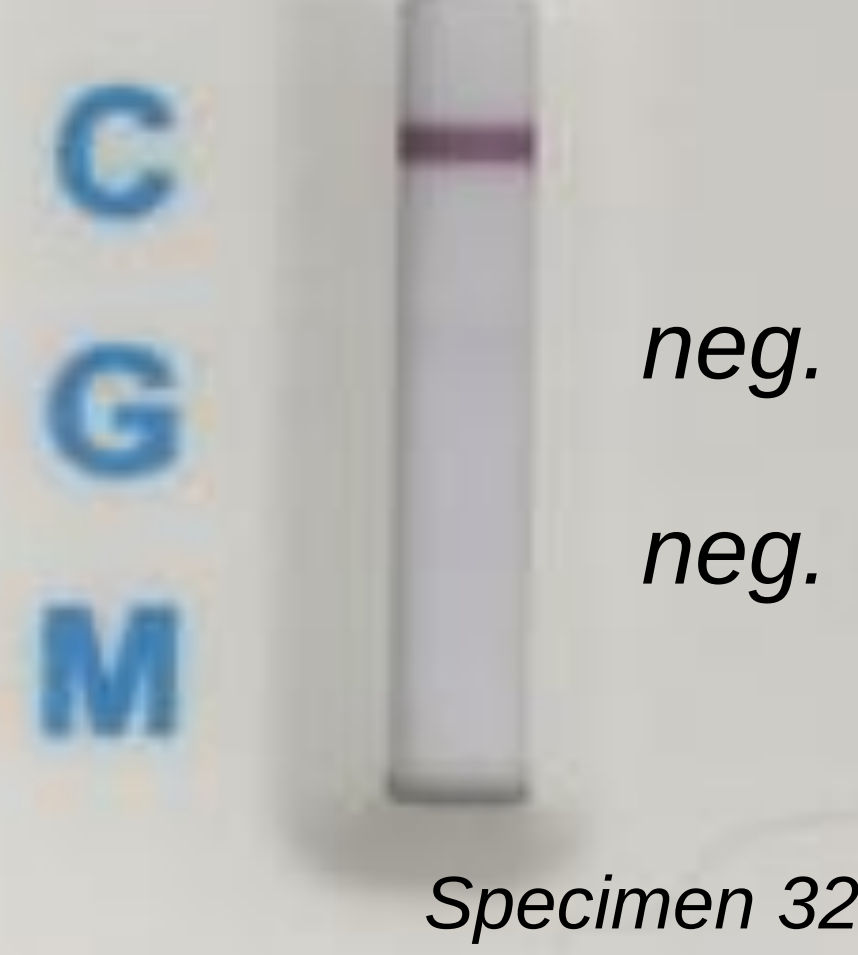
Patient 10 D38



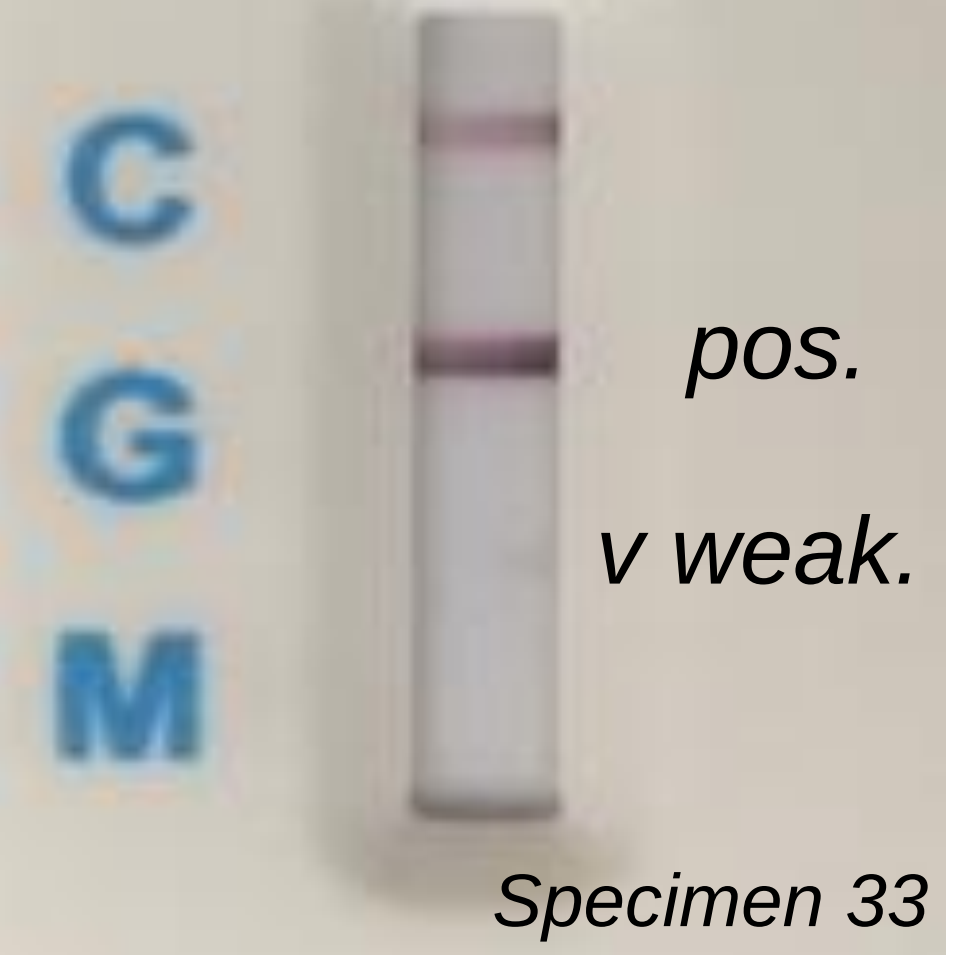
Patient 11 D27



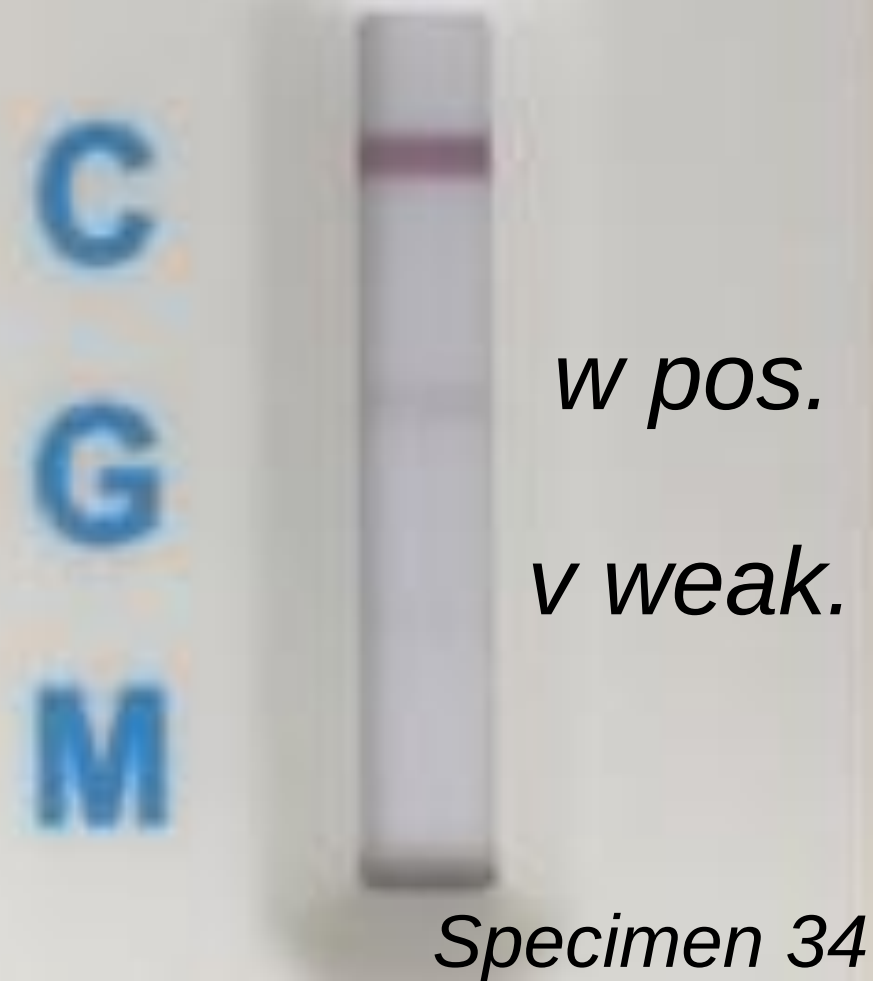
Patient 12 D37



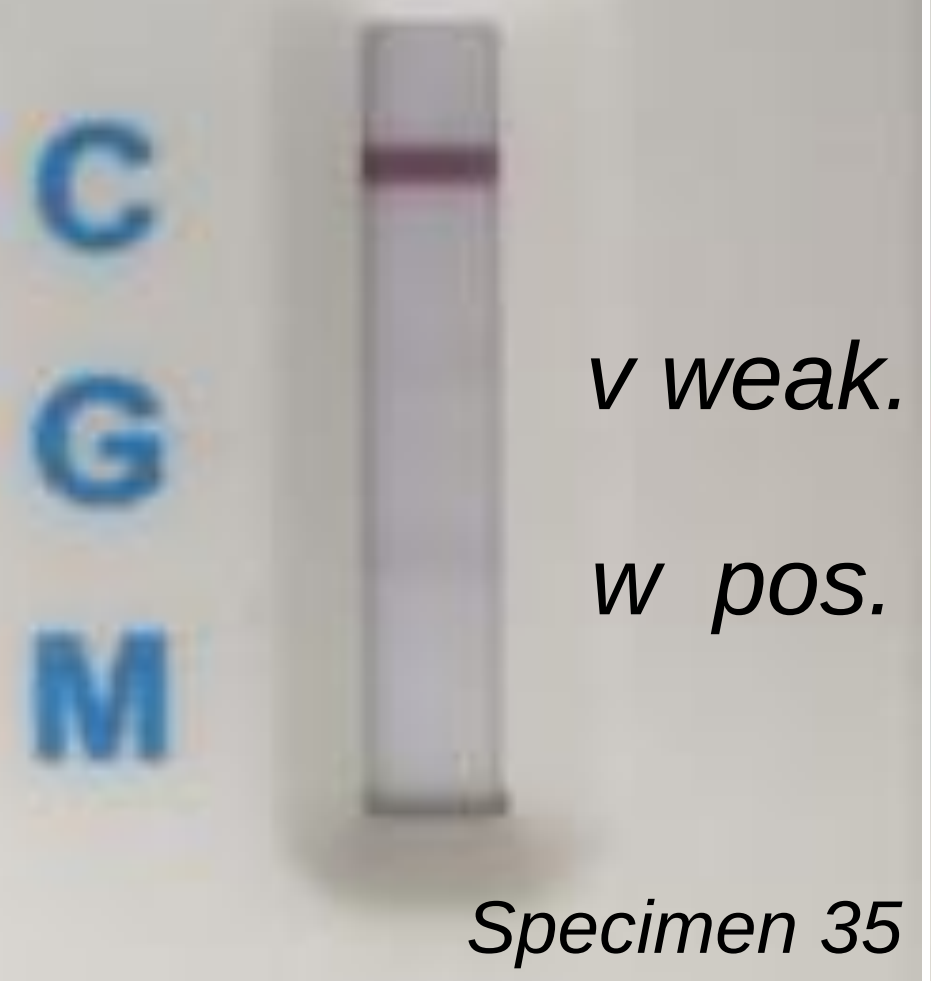
Patient 13 D35



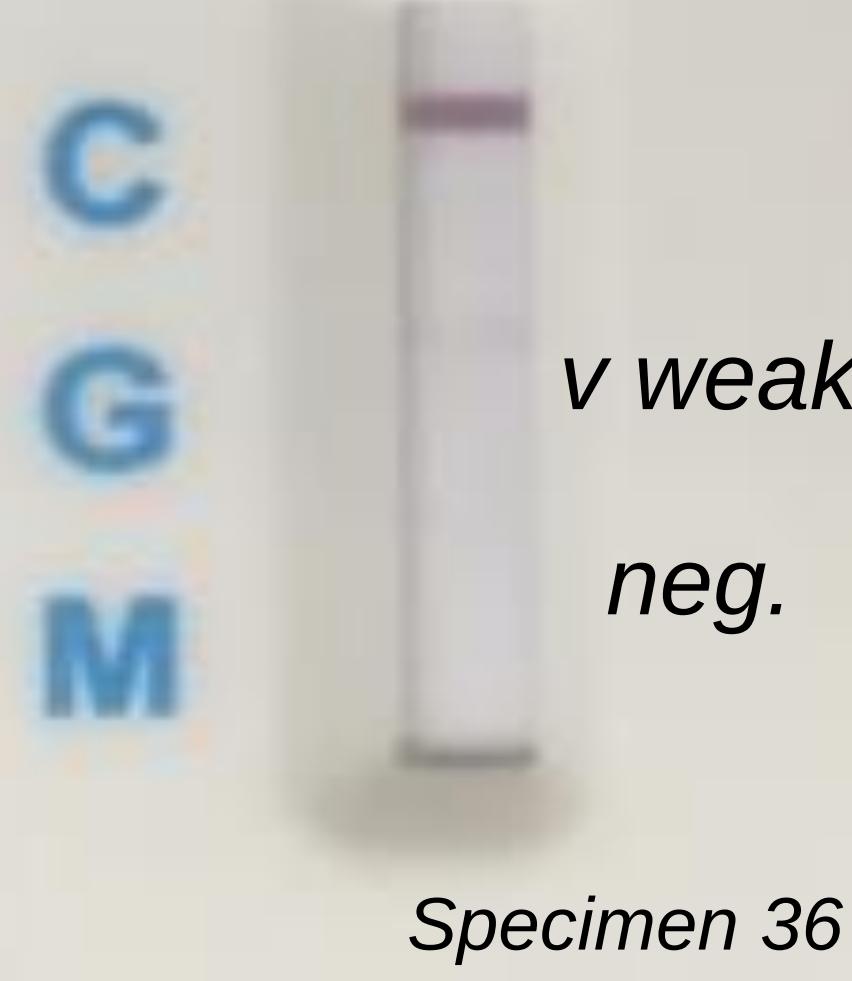
Patient 14 D40



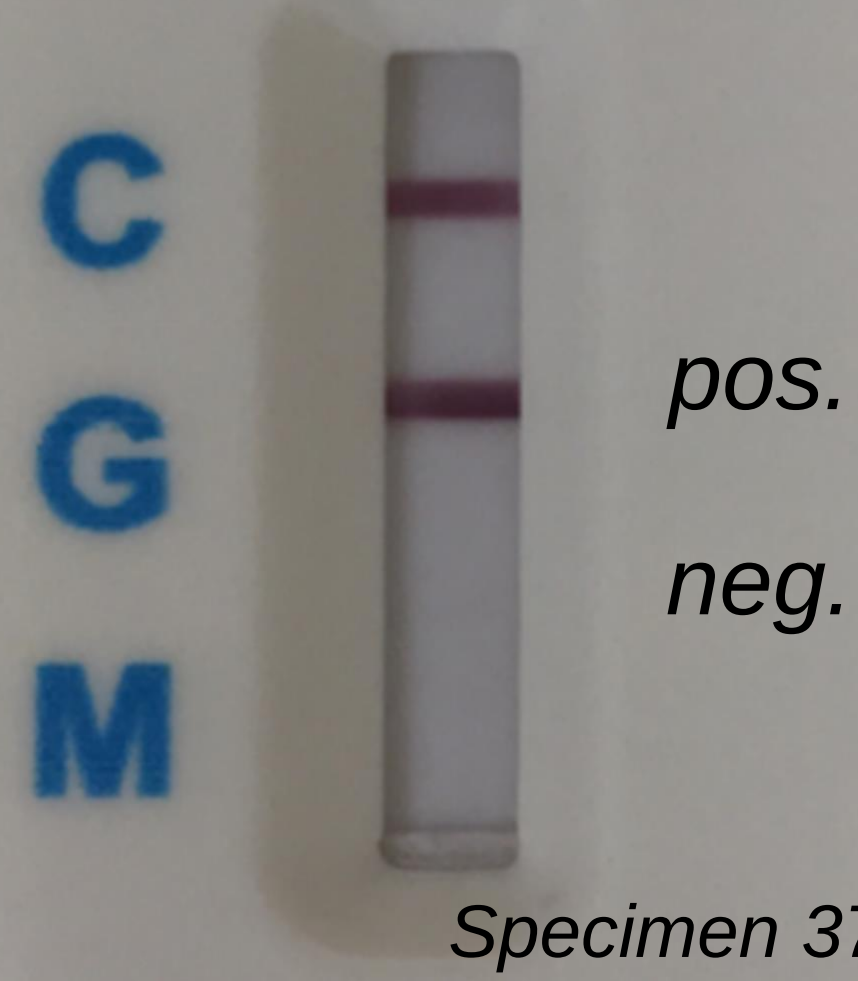
Patient 15 D40



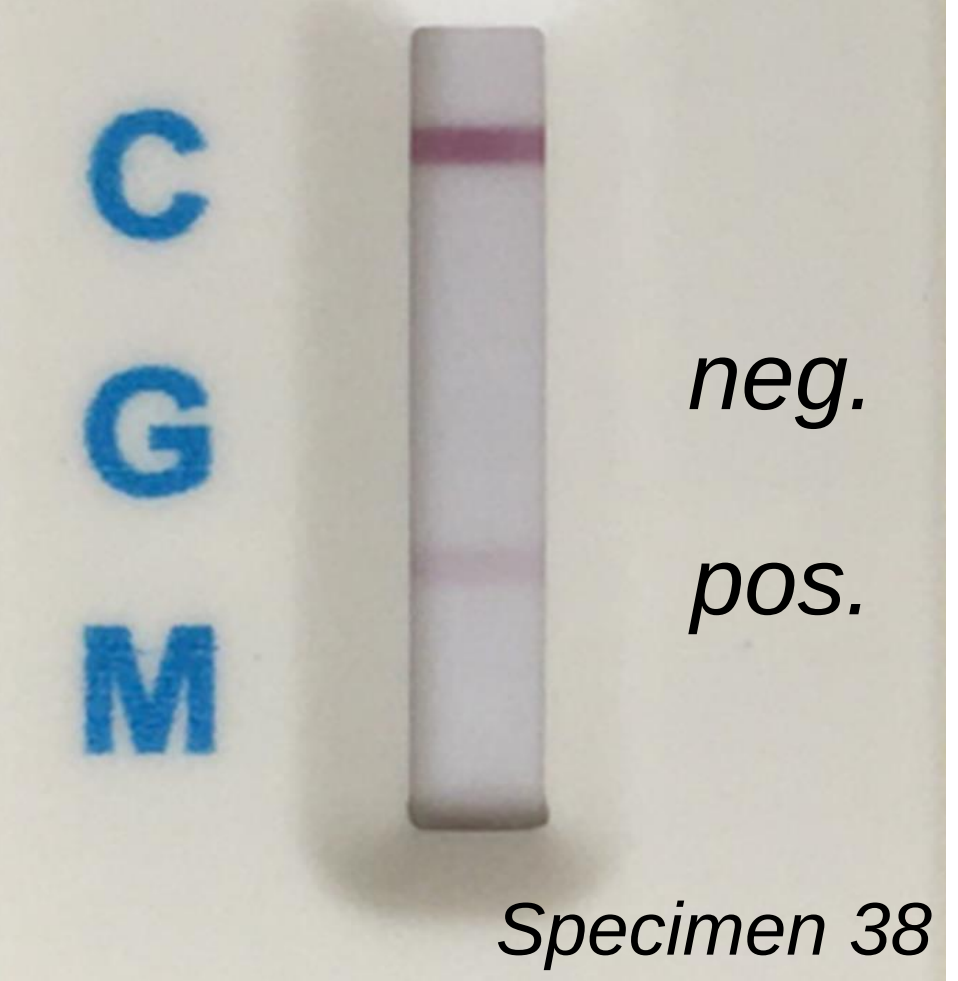
Patient 16 D29



Patient 17 D41



Patient 18 D34



(c) Mild febrile patients (patient 19 – 28)

Serum

Patient 19 D41

C
G
M

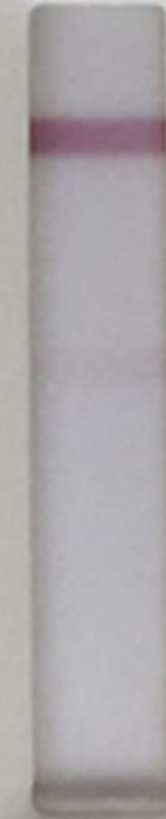


pos.
v weak.

Specimen 39

Patient 20 D42

C
G
M



w pos.
neg.

Specimen 40

Patient 21 D37

C
G
M

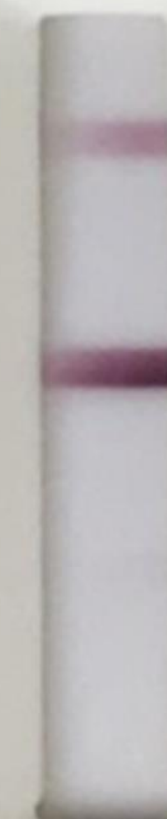


pos.
neg.

Specimen 41

Patient 22 D32

C
G
M

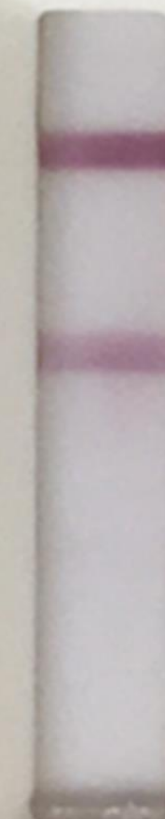


pos.
v weak.

Specimen 42

Patient 23 D47

C
G
M



pos.
neg.

Specimen 43

Patient 24 D56

C
G
M

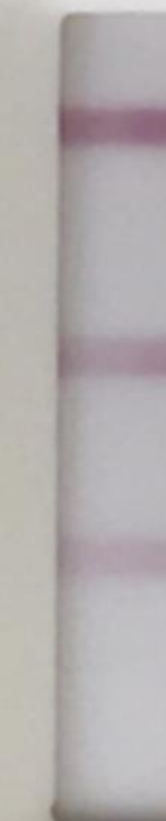


pos.
v weak.

Specimen 44

Patient 25 D53

C
G
M

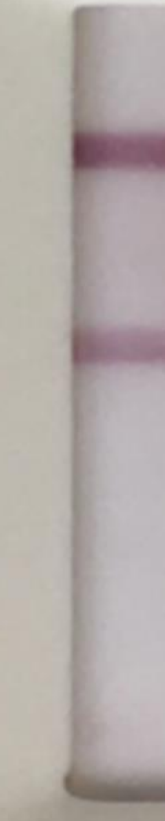


pos.
pos.

Specimen 45

Patient 26 D54

C
G
M

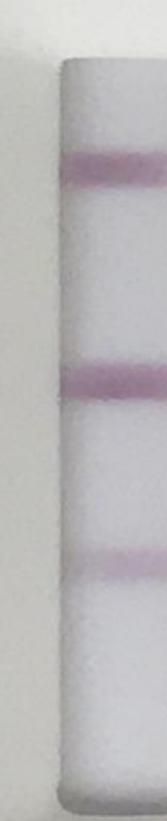


pos.
neg.

Specimen 46

Patient 27 D39

C
G
M



pos.
pos.

Specimen 47

Patient 28 D18

C
G
M

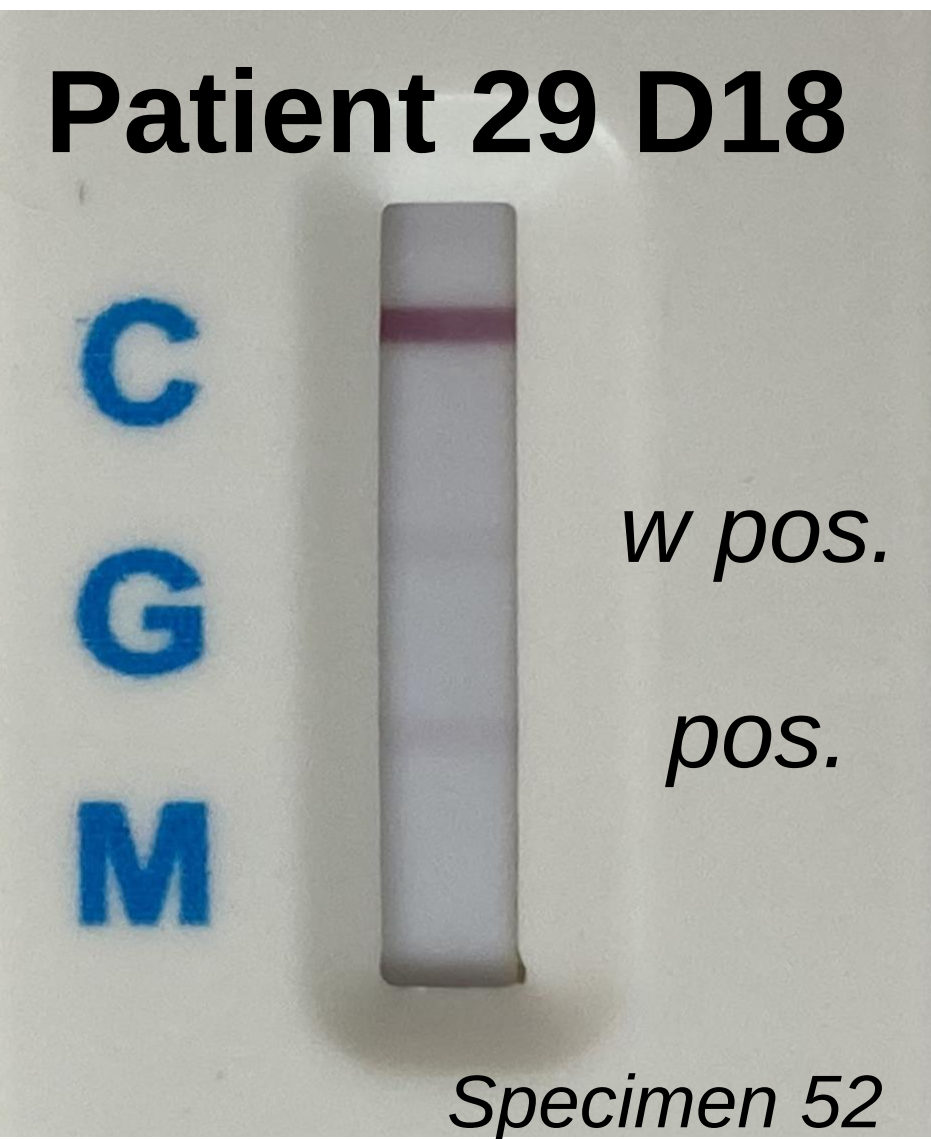


pos.
v weak.

Specimen 48

(c) Mild febrile patients (patient 29)

Serum



(d) Ten-fold titrations

Patient 3 D30

1:1

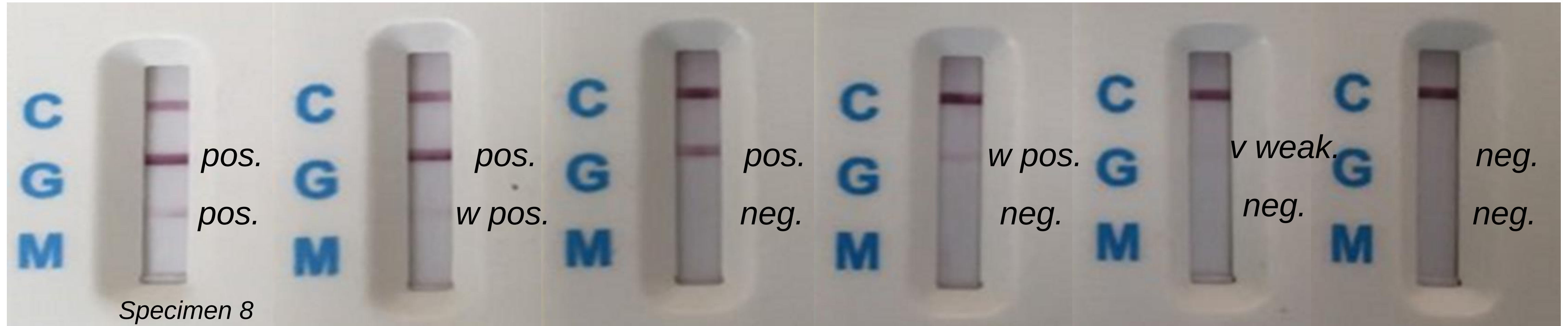
1:10

1:10²

1:10³

1:10⁴

1:10⁵



Patient 4 D42

1:1

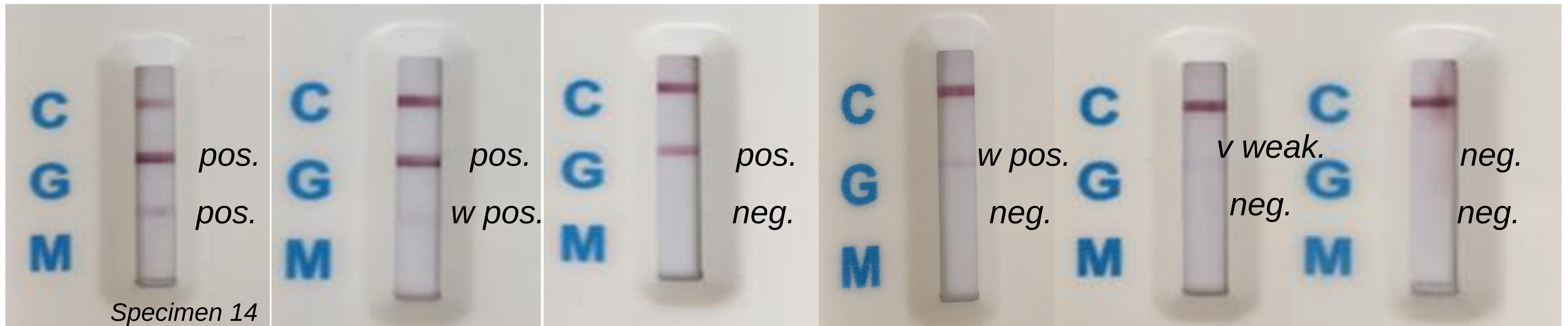
1:10

1:10²

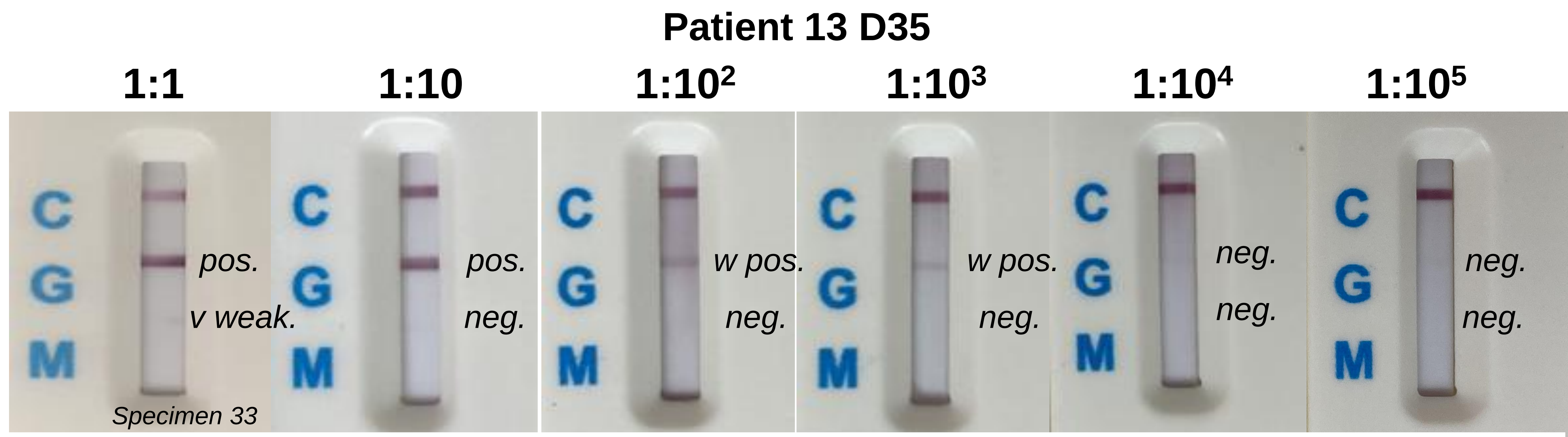
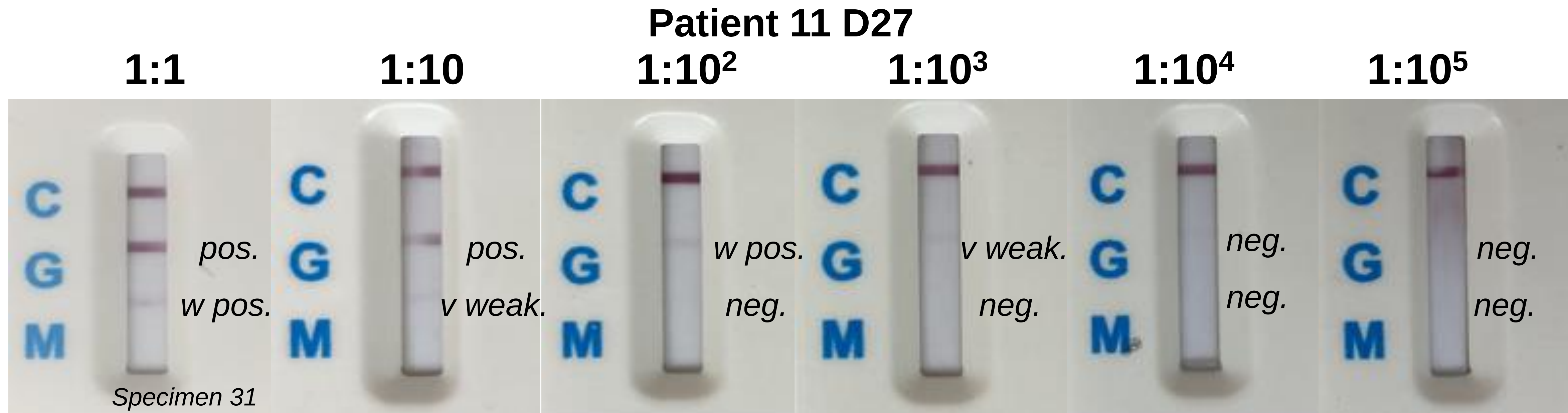
1:10³

1:10⁴

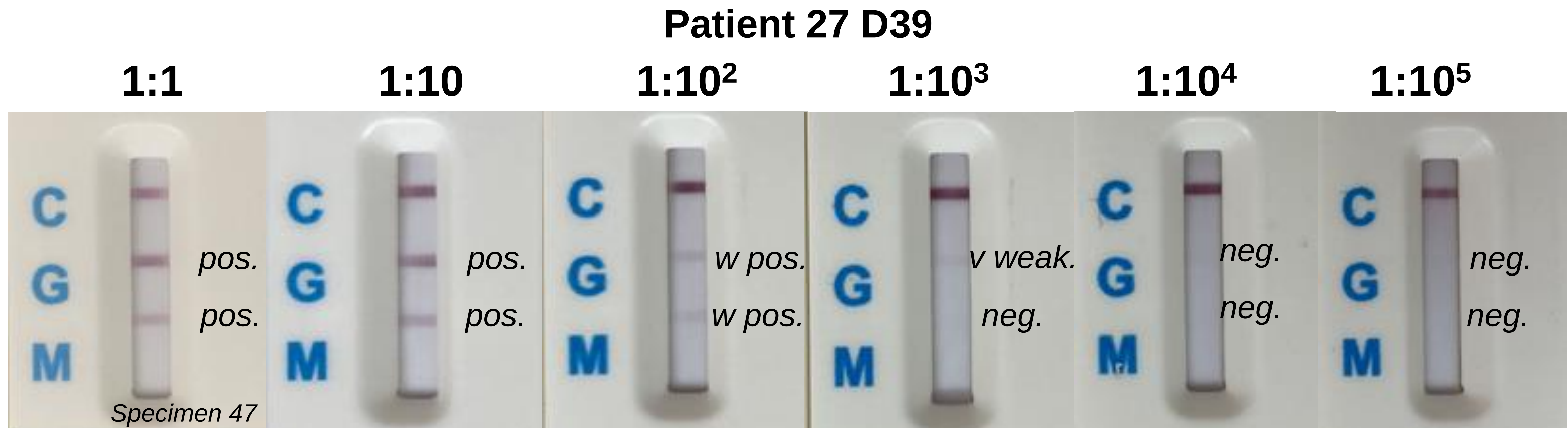
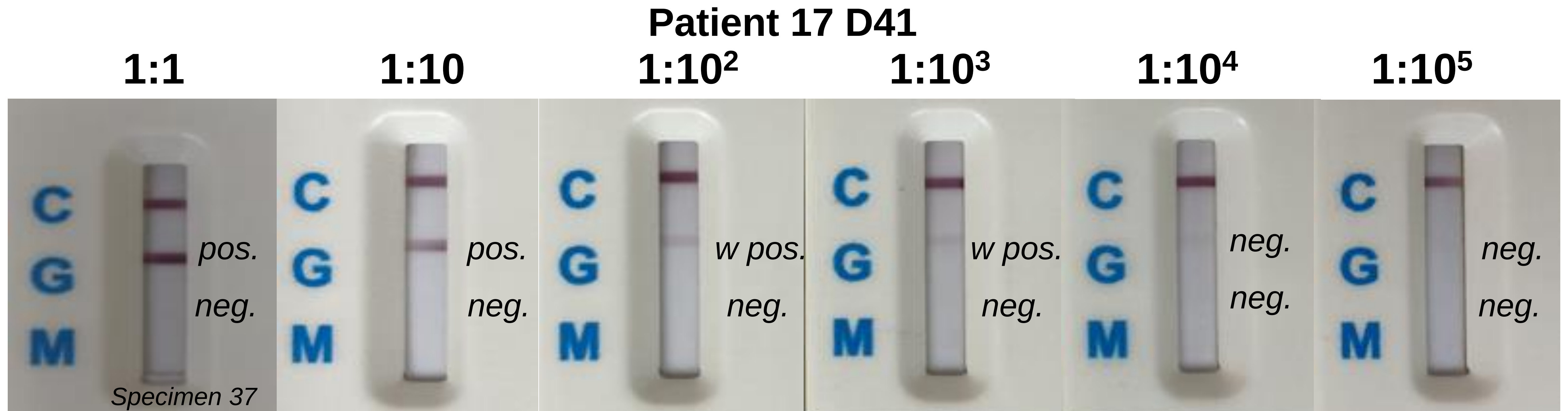
1:10⁵



(d) Ten-fold titrations



(d) Ten-fold titrations



(d) Ten-fold titrations

Patient 28 D18

1:1

1:10

1:10²

1:10³

1:10⁴

1:10⁵

