ms_05909708190V5.0

CMV IgG Avidity

cobas®

IgG antibody avidity to Cytomegalovirus

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
05909708 190	100; equals to 50 CMV IgG avidity determinations	**cobas e** 411
		cobas e 601
		cobas e 602

English

Intended use

Immunoassay for the in vitro qualitative determination of the avidity of IgG antibodies to cytomegalovirus in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Cytomegalovirus, a member of the herpes virus family, is ubiquitous in all human populations, causing infections which are followed by life-long latency in the host with occasional reactivations as well as recurrent infections. CMV infections are usually mild and asymptomatic. However, primary maternal CMV infection during pregnancy carries a risk of intrauterine transmission which may result in severe fetal damage, including growth and mental retardation, jaundice and CNS abnormalities. Those who are asymptomatic at birth may develop hearing defects or learning disabilities later in life. Prenatal CMV infection occurs in approximately 0.2-2.5 % of all life births. Different studies have shown that the risk of symptomatic congenital disease in the fetus or newborn infant is high, when maternal primary infection takes place in early pregnancy before week 20 of gestation, and lower thereafter. The congenital CMV infection caused by non-primary infection seldom leads to congenital disease in the fetus.[1,2,3,4,5]

In the absence of acute clinical manifestation, the diagnosis of CMV infection is usually done by serology. A first step in diagnosing acute CMV infection is most commonly the detection of specific CMV IgG and IgM antibodies. Samples being reactive for CMV IgM antibodies may indicate an acute, recent or reactivated infection. Since symptomatic congenital infection in the fetus is mostly due to intrauterine transmission following primary maternal infection, differential diagnosis of primary versus recurrent infection, polyclonal stimulation or persistence of CMV-specific IgM antibody is crucial for correct counselling and management of pregnancy. The CMV IgG avidity test is currently the most reliable procedure to identify primary infection in pregnant women. The CMV IgG avidity assay measures the functional binding affinity of CMV IgG antibodies in response to infection. The antibodies produced during the primary response have lower antigen avidity than the antibodies produced during the non-primary response or in remote phase of infection. Low avidity is encountered approx. 18-20 weeks after onset of symptoms in immunocompetent subjects. However, individual variation does exist in the rate of avidity maturation. In rare cases low avidity results can be observed up to 6 months or even longer after the onset of infection. The avidity testing should be performed early in gestation. Low avidity CMV IgG antibodies detected before the 16th-18th week of pregnancy in combination with positive CMV IgM result is a strong evidence for recent primary infection. A high avidity result later after gestation (after 20th week of gestation) cannot rule out a primary infection earlier in gestation when low avidity CMV IgG may have been present. A high avidity index during the first 12-16 weeks of pregnancy could be considered as a good indicator of past infection.[6,7,8,9]

Test principle

The test principle is based on two, parallel measurements with the Elecsys CMV IgG Avidity assay.

The first measurement is a reference measurement of the samples with the Elecsys CMV IgG Avidity assay. The second measurement is the DilCMVAv treated measurement of the samples using the automated sample specific dilution function of the analyzer with the avidity diluent (DilCMVAv) followed by the Elecsys CMV IgG Avidity assay. The avidity diluent contains components which interfere with the binding of low avidity CMV IgG antibodies.

The avidity (Avi%) is assessed by determining the ratio between the reference measurement and the DilCMVAv treated measurement.

The Elecsys CMV IgG Avidity assay uses the sandwich principle. Total duration of assay is 18 minutes for both reference measurement and DilCMVAv treated measurement.

- 1st incubation: 20 µL of sample (automatically diluted with DilCMVAv or undiluted reference), biotinylated recombinant CMV-specific antigens, and CMV-specific recombinant antigens labeled with a ruthenium complex[a)] form a sandwich complex. In case of the DilCMVAv treated measurement, only high avidity CMV antibodies are able to build up the sandwich complex, while the complex with low avidity CMV antibodies is dissolved.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as CMV-AV.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 CMV-Ag~biotin (gray cap), 1 bottle, 9 mL:
Biotinylated CMV-specific antigen (recombinant, E. coli), > 400 µg/L, MES buffer 50 mmol/L, pH 6.5; preservative.

R2 CMV-Ag~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
CMV-specific antigen (recombinant, E. coli) labeled with ruthenium complex > 400 µg/L; MES buffer 50 mmol/L, pH 6.5; preservative.

CMV-AV Cal1 Negative calibrator 1 (white cap), 2 bottles of 1.0 mL each:
Human serum, non-reactive for anti-CMV IgG; buffer; preservative.

CMV-AV Cal2 Positive calibrator 2 (black cap), 2 bottles of 1.0 mL each:
Human serum, reactive for anti-CMV IgG, approx. 40 U/mL; buffer; preservative.

DilCMVAv Avidity Diluent (white cap), 1 bottle, 2.5 mL:
0.8 M Guanidine chloride, CMV-specific antigen (recombinant, E. coli); MES-buffer 50 mmol/L, pH 6.5; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

All human material should be considered potentially infectious.

Both calibrators (CMV-AV Cal1, CMV-AV Cal2) have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.

CMV IgG Avidity

IgG antibody avidity to Cytomegalovirus

The serum containing anti-CMV IgG (CMV-AV Cal2) was sterile filtrated.

The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[10,11]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit are ready for use and are supplied in bottles compatible with the system.

Elecsys 2010 and **cobas e** 411 analyzers: The calibrators should only be left on the analyzers during calibration at 20-25 °C. After use, close the bottles as soon as possible and store at 2-8 °C.

Due to possible evaporation effects, not more than 5 calibration procedures per bottle set should be performed.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Unless the entire volume is necessary for calibration on the analyzer, transfer aliquots of the ready-for-use calibrators into empty snap-cap bottles (CalSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots at 2-8 °C for later use.

Perform **only one** calibration procedure per aliquot.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit and DilCMVAv **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagent rackpack and DilCMVAv	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	3 weeks or 8 weeks if stored alternately in the refrigerator and on the analyzers (up to 8 hours at 20-25 °C)

Stability of the calibrators	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on Elecsys 2010 and **cobas e** 411 at 20-25 °C	up to 5 hours
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Only the specimens listed below were tested in a sufficient number and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Criterion: Mean recovery of positive samples within 80-120 % of serum value.

Stable for 4 weeks at 2-8 °C, 7 days at 25 °C, 6 months at -20 °C. The samples may be frozen 5 times.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Specimens should not be subsequently altered with additives (biocides, anti-oxidants or substances that could possibly change the pH of the sample) in order to avoid erroneous findings.

Pooled samples and other artificial material may have different effects on different assays and thus may lead to discrepant findings.

Centrifuge samples containing precipitates and frozen samples before performing the assay. Lyophilized samples and heat-inactivated samples can be used.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- REF 05942322190, PreciControl CMV IgG Avidity, 3 x 1.0 mL each of PreciControl CMV IgG Avidity 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Place the calibrators in the sample zone.

CMV IgG Avidity

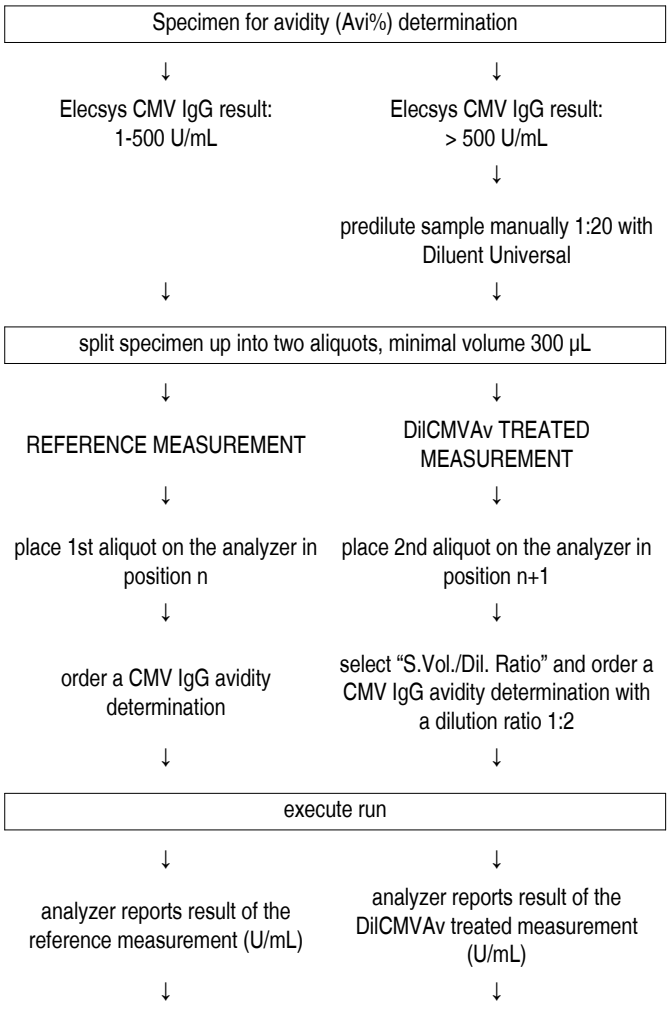
IgG antibody avidity to Cytomegalovirus

All the information necessary for calibrating the assay is automatically read into the analyzer.

After calibration has been performed, store the calibrators at 2-8 °C or discard (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

Each sample and the controls must be ordered twice (reference measurement and DilCMVAv treated measurement) to calculate the avidity (Avi%).

Handling of specimen for the Elecsys CMV IgG Avidity assay



Samples found to be reactive in the Elecsys CMV IgG assay with concentrations between 1-500 U/mL are split up into two aliquots.

If a sample is reactive in the Elecsys CMV IgG assay with a concentration > 500 U/mL, the sample has to be prediluted manually 1:20 with Diluent Universal (refer to the "Dilution" section) and then split up into two aliquots.

- Reference measurement:

  Place first aliquot of a given sample on the analyzer in position n and order a CMV IgG avidity measurement.

- DilCMVAv treated measurement:

  Place second aliquot of the above mentioned sample on the analyzer in position n+1 and order a CMV IgG avidity measurement with a "sample specific dilution" of 1:2. For further details please refer to the analyzer operator's manual. By means of this, the analyzer will mix 50 µL DilCMVAv with 50 µL sample prior to the CMV IgG avidity measurement.

Operator must ensure that both measurements are performed consecutively with the same reagent lot, with the same analyzer and the same calibration.

Note: If aliquoting of a sample is not possible, the two measurements have to be programmed and performed consecutively. Parallel, automated measurement of reference measurement and the DilCMVAv treated measurement from one aliquot is not possible.

Calibration

Traceability: This method has been standardized against the internal Roche standard for CMV IgG. No international standard is available for CMV.

Every Elecsys CMV IgG Avidity reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using CMV-AV Cal1 and CMV-AV Cal2.

Calibration frequency: Calibration must be performed once per reagent lot using CMV-AV Cal1, CMV-AV Cal2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings with PreciControl CMV IgG Avidity outside the defined limits
- more frequently when this is required by pertinent regulations

Quality control

For quality control, use PreciControl CMV IgG Avidity.

It is recommended to run PreciControl CMV IgG Avidity 1 and 2 at the beginning of each working day and after every calibration. Prepare two aliquots of each control level. Place both aliquots of each control level one after another on a sample rack. Both levels must be run in parallel in the reference and in the DilCMVAv treated measurement as a performance check.

Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

- Verification of the calibration:

  The target values and ranges (U/mL) of the reference measurement were determined and evaluated by Roche. They were obtained using the Elecsys CMV IgG Avidity test reagents and analyzers available at the time of testing. The reference measurements of the controls have to be recovered within the control ranges (U/mL) as stated in the value sheet. The control values have to be compared manually to the CMV IgG ranges (U/mL) given in the value sheet. The exact lot-specific target values and ranges are printed on the enclosed (or electronically available) value sheet.

- Verification of the functionality of the Diluent Avidity (DilCMVAv):

  The avidity (Avi%) is calculated from the reference measurement and the DilCMVAv treated measurement according to the "Calculation" section. The target range for the manually calculated avidity result (Avi%) of PreciControl CMV IgG Avidity 1 is < 45.0 Avi%, while the respective range for PreciControl CMV IgG Avidity 2 is ≥ 55.0 Avi%.

Note: The controls are not barcode-labeled and have to be treated as patient samples.

Controls must not be defined as external controls, as dilution of controls is not possible on the instrument.

Calculation

The analyzer automatically calculates the analyte concentration of each sample in U/mL for both measurements (reference measurement and DilCMVAv treated measurement). The avidity (Avi%) must be calculated manually:

$$\text{Avi\%} = \frac{\text{result DilCMVAv treated measurement}}{\text{result reference measurement}} \times 100\ \%$$

Only samples being reactive in the reference measurement (≥ 1.0 U/mL) can be used for the determination of avidity (Avi%).

If the DilCMVAv treated measurement results in values < 0.3 U/mL, please calculate avidity (Avi%) with 0.3 U/mL.

Interpretation of the results

CMV IgG Avidity

IgG antibody avidity to Cytomegalovirus

Results obtained with the Elecsys CMV IgG Avidity assay are interpreted as follows:

Avidity	Interpretation
< 45.0 Avi%	low avidity
45.0-54.9 Avi%	gray-zone
≥ 55.0 Avi%	high avidity

No clinical interpretation can be deduced from a gray-zone result. It is recommended to take a follow-up sample within an appropriate period of time (e.g. 2-4 weeks) and repeat testing.

Elecsys CMV IgG Avidity results should be used in conjunction with the patient's medical history, clinical symptoms and other laboratory tests, e.g. CMV-specific IgG and IgM results.

In case of a CMV IgG avidity result discordant to the patient's medical history, clinical symptoms and other laboratory tests, e.g. CMV-specific IgG and IgM results, further tests should be performed to verify the result and testing of a follow up sample is recommended.

The CMV IgG avidity results in a given specimen, as determined by assays from different manufacturers, can vary due to differences in assay methods and reagents used. Therefore, the results reported by the laboratory to the physician should include: "The following results were obtained with the Elecsys CMV IgG Avidity assay. Results from assays of other manufacturers cannot be used interchangeably."

Avidity results up to 110 Avi% can occur due to the assay inherent variance and are interpreted as high avidity results. For avidity > 110 Avi%, it is advised to predilute (according to the "Dilution" section) the sample and repeat both measurements to calculate a new avidity (Avi%).

Limitations - interference

The results in HIV patients, in patients undergoing immunosuppressive therapy, or in patients with other disorders leading to immune suppression, should be interpreted with caution.

Specimens from neonates, cord blood, pretransplant patients or body fluids other than serum and plasma, such as urine, saliva or amniotic fluid have not been tested.

Among a panel of 142 positive samples within the measuring range no high-dose hook effect was observed (no increasing signals upon dilution). However, the occurrence of a high-dose hook effect cannot be excluded in other cohorts.

The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), hemolysis (Hb < 0.310 mmol/L or < 0.500 g/dL), lipemia (Intralipid < 2000 mg/dL) and biotin (< 246 nmol/L or < 60 ng/mL).

Criterion: Mean recovery of positive samples within ± 20 % of serum value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

In vitro tests were performed on 18 commonly used pharmaceuticals and in addition on ganciclovir and valganciclovir. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

• Reference measurement:

0.25-500 U/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Blank are reported as < 0.15 U/mL. Values above the Limit of Blank but below the Limit of Detection will not be flagged by the instrument. Values above the measuring range are reported as > 500 U/mL.

Limit of Blank (LoB) and Limit of Detection (LoD)

Limit of Blank = 0.15 U/mL

Limit of Detection = 0.25 U/mL

The Limit of Blank and Limit of Detection were determined in accordance with CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

• DilCMVAv treated measurement:

Due to the 1:2 dilution measuring range is 0.50-1000 U/mL. Values below the Limit of Blank are reported as < 0.30 U/mL. Values above the Limit of Blank but below the Limit of Detection will not be flagged by the instrument. Values above the measuring range are reported as > 1000 U/mL.

Dilution

Samples with CMV IgG concentrations above the measuring range must be prediluted with Diluent Universal prior to testing with the Elecsys CMV IgG Avidity assay. The recommended predilution is 1:20 (manually). The concentration of the prediluted sample must be ≥ 15 U/mL. This manual predilution must not be considered for avidity (Avi%) calculation as the manually prediluted sample is used for both measurements (reference measurement and DilCMVAv treated measurement).

Note: Antibodies to CMV are heterogeneous. This may lead to non-linear dilution behavior.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, human samples and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84); repeatability n = 21. The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean Avi%	SD Avi%	CV %	Mean Avi%	SD Avi%	CV %
Human serum 1	9.8	0.1	1.0	9.8	0.2	2.3
Human serum 2	50.0	0.8	1.5	50.0	1.0	2.0
Human serum 3	81.1	1.4	1.7	81.1	1.8	2.2
PC[b)] CMV IgG Avidity 1	28.5	0.6	1.9	28.5	0.9	3.1
PC CMV IgG Avidity 2	78.1	1.0	1.3	78.1	1.7	2.2

b) PC = PreciControl

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean Avi%	SD Avi%	CV %	Mean Avi%	SD Avi%	CV %
Human serum 1	9.4	0.1	1.2	9.4	0.3	3.4
Human serum 2	53.1	0.8	1.5	53.1	0.9	1.6
Human serum 3	85.0	1.1	1.3	85.0	1.5	1.7
PC CMV IgG Avidity 1	29.6	1.0	3.4	29.6	1.1	3.8
PC CMV IgG Avidity 2	79.5	1.1	1.4	79.5	1.2	1.5

Analytical specificity

439 potentially cross reacting samples were tested with the Elecsys CMV IgG assay (which equals to the Elecsys CMV IgG Avidity reference

CMV IgG Avidity

IgG antibody avidity to Cytomegalovirus

measurement) and a comparison CMV IgG assay comprising the following specimens:

- containing antibodies against HBV**, HAV, HCV*, HIV, HTLV, EBV**, HSV*, VZV**, Parvo B19***, Rubella, Treponema pallidum**, Toxoplasma gondii**
- containing autoantibodies*** (ANA, anti-tissue, RF)

An overall agreement of 96.6 % (422/437) was found in these specimens with the Elecsys CMV IgG Avidity reference measurement and the comparison test. 110 samples were found concordantly negative and 312 samples were found positive. 2 samples were found indeterminate either with the Elecsys CMV IgG assay or the comparison test.

* HSV, HCV: 2 discordant samples were found in each group.

** HBV, EBV, VZV, Treponema pallidum, Toxoplasma gondii: 1 discordant sample was found in each group.

*** Parvo B19, autoantibodies: 3 discordant samples were found in each group.

Sensitivity

Sensitivity (concordance of low avidity results with primary infections):

The sensitivity of the CMV IgG avidity assay is defined as the percentage of samples of CMV primary infections (characterized by reference laboratories) detected to contain low avidity CMV IgG antibodies.

Overall 183 single and sequential samples collected by reference laboratories and characterized (based on diagnostic testing and if available, clinical indications) to be from primary CMV infections were investigated. 31 samples showed a gray-zone result and were excluded from calculation.

Sample type	Sensitivity (%)	Lower 95 % confidence limit (%)	Upper 95 % confidence limit (%)
Diagnostic	96.1 (74/77)	89.0	99.2
Pregnant women	93.4 (99/106)	86.9	97.3
Total	94.5 (173/183)	90.2	97.4

Relative sensitivity (concordance of low avidity results to two commercial CMV IgG avidity assays):

Single specimens from randomly selected blood donor samples with CMV IgG seroconversion from the previous to the actual donation and characterized to contain CMV IgG low avidity antibodies with two commercial CMV IgG avidity assays were investigated. In 22 samples out of 24 samples low avidity CMV IgG antibodies were detected. 1 sample showed a gray-zone result.

Specificity

Specificity (concordance of high avidity results with late infections):

The specificity of the CMV IgG avidity assay is defined as the percentage of samples of CMV late infections (characterized by reference laboratories) detected to contain high avidity CMV IgG antibodies.

A total of 95 single samples collected by a reference laboratory and characterized (based on diagnostic testing) to be from late CMV infections were investigated.

12 samples showed a gray-zone result and were excluded from calculation.

Sample type	Specificity (%)	Lower 95 % confidence limit (%)	Upper 95 % confidence limit (%)
Diagnostic	90.9 (40/44)	78.3	97.5
Pregnant women	100 (51/51)	93.0	100
Total	95.8 (91/95)	89.6	98.8

Relative specificity (concordance of high avidity results in CMV IgG reactive, CMV IgM non-reactive samples indicating the absence of a primary infection):

A total of 365 samples from blood donor testing and pregnancy screening (calculated from CMV IgG reactive, CMV IgM non-reactive samples with concordant high avidity results in two comparison methods indicating the absence of a primary infection) were investigated. 20 samples showed a gray-zone result and were excluded from calculation.

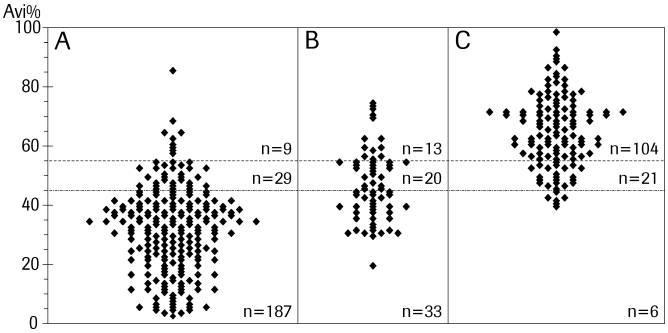
Sample type	Relative specificity (%)	Lower 95 % confidence limit (%)	Upper 95 % confidence limit (%)
Blood donors	98.5 (130/132)	94.6	99.8
Pregnant women	100 (233/233)	98.4	100
Total	99.5 (363/365)	98.0	99.9

Distribution of avidity

The ability to discriminate between acute and late CMV infection is shown with 422 single and sequential samples collected by reference laboratories and classified into one of the following categories:

- Category A: < 90 days after presumed onset of infection or date of first bleed (single and sequential samples of patients initially suffering from a CMV primary infection); n = 225 samples
- Category B: 90-180 days after presumed onset of infection or date of first bleed (single and sequential samples of patients initially suffering from a CMV primary infection); n = 66 samples
- Category C: > 180 days after presumed onset of infection or date of first bleed (single and sequential samples of patients initially suffering from a CMV primary infection); n = 131 samples

The exact distribution of low avidity, gray-zone and high avidity results is given in the following diagram:



References

1 Boppana SB, Pass RF, Britt WS, et al. Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality. Pediatr Infect Dis J 1992;11:93-99.

2 Alford CA, Stagno S, Pass RF, et al. Congenital and perinatal cytomegalovirus infections. Rev Infect Dis 1990;12:745-753.

3 Peckham CS. Cytomegalovirus infection: congenital and neonatal disease. Scand J Infect Dis Suppl 1991;80:82-87.

4 Fowler KB, Stagno S, Pass RF, et al. The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. N Eng J Med 1992;326:663-667.

5 Pass RF, Fowler KB, Boppana SB, et al. Congenital cytomegalovirus infection following first trimester maternal infection: symptoms at birth and outcome. J Clin Virol 2006;35:216-220.

6 Revello MG, Gerna G. Maternal, fetal and neonatal diagnosis of congenital human cytomegalovirus infection. Expert Opin Med Diagn 2008;2(5):547-563.

7 Grangeot-Keros L, Mayaux MJ, Lebon P, et al. Value of cytomegalovirus (CMV) IgG avidity index for the diagnosis of primary infection in pregnant women. J Infect Dis 1997;175:944-946.

8 Macé M, Sissoeff L, Rudent A, et al. A serological testing algorithm for the diagnosis of primary CMV infection in pregnant women. Prenat Diagn 2004;24:861-863.

9 Lazzarotto T, Guerra B, Lanari M, et al. New advances in the diagnosis of congenital cytomegalovirus infection. J Clin Virol 2008;41:192-197.

10 Occupational Safety and Health Standards: bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

11 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com