Elecsys CMV IgG Avidity

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REF		Σ	SYSTEM
09342397190	09342397500	100; equals to 50 CMV IgG avidity determinations	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 640
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 030

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 **cobas e** immunoassay analyzers.

Summary

Cytomegalovirus (CMV), 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.[1,2] The seroprevalence of antibodies in adults ranges from 40-100 % with inverse correlation to socioeconomic status.[1,2,3] CMV is transmitted through body fluids, including blood, genital secretions and breast milk. Saliva and urine of infected individuals also represent a prominent source of infection, and children, especially those attending day care facilities, are an important vector for viral spread.[2,3,4,5,6] In immunocompetent individuals primary CMV infection is usually mild or asymptomatic.[2,5] Patients commonly present with a mononucleosis-like syndrome, including fever, sore throat, cervical lymphadenopathy, malaise, headache, muscle ache and joint pains.[2,3,4,5,7]

During pregnancy, CMV can cause congenital infection which may result in permanent physical and/or neurological sequelae in the child.[5] CMV infection can be primary, i.e. newly acquired, or secondary, i.e. due to reactivation of the latent virus or re-infection with a different viral strain.[3,5] Primary CMV infection is reported in 1-4 % of seronegative women during pregnancy and the risk of transmission to the fetus is estimated to be about 30-40 %.[3,4] Reactivation of CMV infection during pregnancy is reported in 10-30 % of seropositive women and, in this circumstance, the risk of transmission of the virus is about 1-3 %.[3,4,5] Overall, prenatal CMV infection occurs in 0.6-0.7 % of all life births in the developed world.[4,5,8] The majority of babies born with congenital CMV infection are asymptomatic at birth.[8,9,10] Of these 5-15 % still develop irreversible impairments, most frequently hearing loss, that can occur several months or even years after birth.[5,8,9,10] For babies symptomatic at birth, prognosis is very poor, as they are likely to develop severe mental impairment and/or hearing loss.[5,8,9,10] 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.[4,5] The congenital CMV infection caused by recurrent maternal infection seldom leads to symptomatic disease at birth.[4,5]

At risk for CMV infection and disease are also immunocompromised patients such as transplant recipients and HIV infected patients where the virus can cause life-threatening diseases.[11,12] The CMV status of transplant donors and recipients is very important, as it will determine prophylactic and pre-emptive treatment strategies against CMV. CMV-negative transplant recipients should receive donations from CMV-negative individuals or leukocyte depleted blood products. During latency, CMV resides in infected cells and the free viral DNA load is usually low. The CMV status can still be determined by testing for CMV IgG antibodies.

Within the appropriate clinical context, the first step in diagnosing acute primary CMV infection is most commonly made by the detection of anti-CMV-specific IgG and IgM antibodies.[5] Samples being reactive for IgM antibodies indicate an acute, recent or reactivated infection.[2,4,5,12] For further analysis of a primary CMV infection the determination of the CMV IgG avidity is used as an aid.[2,4,5,12] 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 later on.[2,5,10] Low avidity is encountered approximately up to 18-20 weeks after onset of symptoms in immunocompetent subjects.[5,10] 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.[3,5,10] 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.[3] A high avidity index during the first 12-16 weeks of pregnancy can be considered indicative of past infection.[3,5,7,10]

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 or e-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.

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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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.
Prevention:	
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.
Response:	
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.
Disposal:	
P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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.

The serum containing anti-CMV IgG (CMV-AV Cal2) was 0.2 micron filtrated.

The testing methods used assays approved by the FDA 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.[13,14]

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.

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

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

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.

Please note for **cobas e** 602 analyzers: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. Please turn the vial cap 180° into the correct position so that the barcode between the yellow markers can be read by the system. Place the vial on the analyzer as usual.

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 **cobas e** 411 at 20-25 °C	up to 5 hours
on **cobas e** 601 and **cobas e** 602 at 20-25 °C	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 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 7 days at 20-25 °C, 28 days at 2-8 °C, 6 months at -20 °C (± 5 °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 (e.g. biocides, anti-oxidants or substances that could possibly change the pH or ionic strength 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 thawed 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, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

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Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- REF 04784600190, PreciControl CMV IgG, 16 x 1.0 mL
- REF 05942322190, PreciControl CMV IgG Avidity, 6 x 1.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or
  REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent or
  REF 05192943190, Diluent Universal 2, 2 x 36 mL sample diluent
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **cobas e** 411 analyzer:

- 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, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **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, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Additional materials 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.

Calibrators:

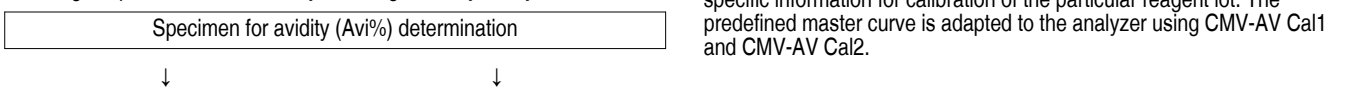
Place the calibrators in the sample zone.

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 (**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 or Diluent Universal 2 (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.

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.

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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).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

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 outside the defined limits

Quality control

For quality control, use PreciControl CMV IgG and PreciControl CMV IgG Avidity.

It is recommended to run PreciControl CMV IgG 1 and 2 as well as 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:

  PreciControl CMV IgG serves to verify 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):

  PreciControl CMV IgG Avidity serves to verify the functionality of the Diluent Avidity.

  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

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 effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.310 mmol/L or ≤ 500 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1500 IU/mL

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

In vitro tests were performed on 17 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 and Limit of Detection

Limit of Blank = 0.15 U/mL

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Limit of Detection = 0.25 U/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 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 or Diluent Universal 2 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, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer						
	Repeatability			**Intermediate precision**		
Sample	Mean Avi%	SD Avi%	CV %	Mean Avi%	SD Avi%	CV %
Human serum 1	32.9	0.674	2.0	32.9	0.678	2.1
Human serum 2	51.7	1.85	3.6	51.7	1.94	3.8
Human serum 3	69.6	1.13	1.6	69.6	1.35	1.9
PC[b)] CMV IgG Avidity 1	27.6	0.456	1.6	27.6	0.757	2.7
PC CMV IgG Avidity 2	82.6	1.05	1.3	82.6	1.38	1.7

b) PC = PreciControl

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	Repeatability			**Intermediate precision**		
Sample	Mean Avi%	SD Avi%	CV %	Mean Avi%	SD Avi%	CV %
Human serum 1	32.3	1.05	3.3	32.3	1.08	3.4
Human serum 2	49.5	1.15	2.3	49.5	1.27	2.6
Human serum 3	68.4	1.15	1.7	68.4	1.28	1.9
PC CMV IgG Avidity 1	26.3	0.501	1.9	26.3	0.559	2.1
PC CMV IgG Avidity 2	82.3	1.09	1.3	82.3	1.17	1.4

Analytical specificity

439 potentially cross reacting samples were tested with the Elecsys CMV IgG assay (which equals to the Elecsys CMV IgG Avidity reference 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 182 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. 32 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 (73/76)	88.9	99.2
Pregnant women	93.4 (99/106)	86.9	97.3
Total	94.5 (172/182)	90.1	97.3

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

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Sample type	Specificity %	Lower 95 % confidence limit %	Upper 95 % confidence limit %
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.

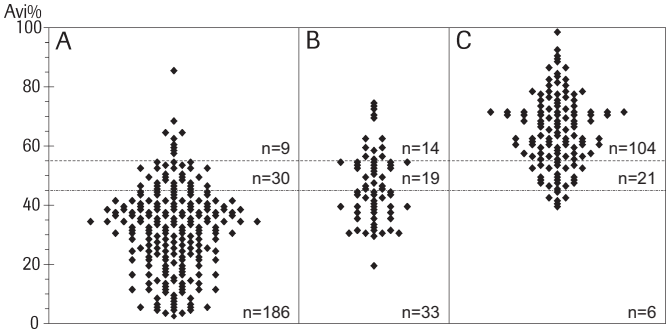
Sample type	Relative specificity %	Lower 95 % confidence limit %	Upper 95 % confidence limit %
Blood donors	98.5 (130/132)	94.6	99.8
Pregnant women	100 (233/233)	98.4	100
Total	99.5 (363/365)	98.0	99.9

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:



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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets 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 (for USA: see dialog.roche.com for definition of symbols used):

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

CE 0123

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