COMPLEMENT C3

Diagnostic reagent for determination of Complement C3 concentration.
Liquid. Dual reagent. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Pack
MH-412	75 mL
MH-413	50 mL
VH-412	75 mL
VH-413	50 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of Complement C3 in serum and plasma.

GENERAL INFORMATION

Complement 3 (C3) is a central molecule in the complement system and its activation is essential for all important functions performed by this system. C3 is the most abundant protein in serum, belonging to the complement system; it is composed of α and β chains linked together by covalent (a single disulfide bond) and non-covalent forces.[1] The protein is encoded by a 41 kb gene located on chromosome 19.[2] Although predominantly synthesized in the liver, it is also synthesized by monocytes-macrophages,[3,4] neutrophils,[5] T cells, antigen presenting cells (APCs),[6] dendritic cells[7] and various other cell types of mesenchymal origin.[8]

C3 is an important component of the innate immune system that forms together with other complement proteins for the detection and clearance of potential pathogens.[9] This large molecular weight protein (185 kDa) interacts with at least 25 different soluble and membrane-bound proteins and is involved in all three pathways of complement activation: classical, alternative and lectin pathway.[8]

Overproduction and premature activation of the complement system can trigger an autoimmune response in which the body's immune system attacks its own tissues and organs. The resulting damage can be cumulative, as in rheumatoid arthritis (RA) and multiple sclerosis, or acute, such as the complete destruction of pancreatic islet cells in type 1 diabetes. Deficiencies in the production of complement factors can make a person susceptible to the emergence and spread of bacterial, fungal or viral infections.[10]

The main function of the complement system is to recruit effector phagocytes for opsonization and clearance of foreign pathogens and to trigger direct destruction of foreign organisms.[11]

There is also a growing amount of scientific work on their pathophysiological contribution to the development of inflammatory, autoimmune, hematologic and nephrologic diseases; studies have also demonstrated their etiologic links to various diseases, including cancer and neurodegenerative disorders.[12-15] For example, deficiencies of components of the classical pathway lead to the development of autoimmune disorders [e.g. RA, systemic lupus erythematosus (SLE) and vasculitis] and render individuals susceptible to recurrent respiratory infections and infections caused by encapsulated organisms.[16] Excessive complement activation has also been described in many disease states such as hereditary/acquired angioedema, Alzheimer's syndrome, asthma, cerebral embolism, Crohn's disease, depression, glaucoma, Huntington's disease, myocardial infarction, various types of kidney disease [e.g., immune complex glomerulonephritis, hemolytic uremic syndrome (HUS), lupus nephritis, membranous nephritis, IgA nephropathy], malnutrition, resistant arterial hypertension, septicemia and serum sickness.[17] The synthesis of C3, an acute phase reactant, is increased in acute inflammatory states.[8] High serum concentrations of C3, which is also defined as a specific marker of chronic inflammation, may provide important tips about the progression of atherosclerosis.[18] In one study, elevated serum C3 levels were associated with an increased risk of adverse clinical outcomes 3 months after ischemic stroke, suggesting that serum C3 levels may be a valuable prognostic biomarker for ischemic stroke .[38] In another study, it was reported that elevated C3 levels were associated with poor outcomes in patients with Guillain-Barré syndrome.[39] In addition, insulin resistance, diabetes, metabolic syndrome,[19-21] atopic dermatitis,[22] rheumatic disease, viral hepatitis, myocardial infarction, cancer, pregnancy, sarcoidosis, amyloidosis, thyroiditis, inflammatory bowel disease, typhoid fever and pneumococcal pneumonia have been associated with high C3 levels.[23]

Low serum C3 is observed in complement deficiency, glomerulonephritis, lupus erythematosus and sepsis.[11] Congenital deficiency of C3 is possible. C3 levels decrease in immunologic diseases due to high consumption of complement factors. C3 levels also decline in acute and chronic hypocomplementemic nephritis, infective endocarditis, partial lipodystrophy (with associated nephritis-like activity in serum). Hereditary cases of C3 deficiency are characterized by recurrent infection and immune complex disease, particularly membranoproliferative glomerulonephritis. There is a risk of severe infection with encapsulated bacteria such as S.

pneumoniae, H. influenzae and N. meningitidis. Bacteremia, sinopulmonary infections, meningitis, paronychia and impetigo may occur. Low seum C3 levels have also been found in uremia, chronic liver diseases, anorexia nervosa and celiac disease.[24] Low C3 levels in HUS have been reported as a reflection of complement activation and depletion.[8] It is stated that complement factor deficiencies may play an active role in the pathogenesis of SLE and complete C3 deficiency is associated with SLE-like disease.[25] In another study, decreased serum C3 levels were associated with impaired renal function in patients with IgA nephropathy.[26] Similarly, low serum C3 levels have been associated with poor prognosis in the diagnosis of renal anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis; in addition, studies have demonstrated the key role of the alternative pathway of complement in the pathophysiology of ANCA-associated vasculitis.[27]

TEST PRINCIPLE

Immunoturbidimetric measurement

In the presence of anti-human C3 antibodies, complement C3 in the sample precipitates. The turbidity of the antibody-antigen complex formed as a result of this precipitation is measured by absorbance reading at 340 nm wavelength and is proportional to the concentration of C3 in the sample.

REAGENT COMPONENTS

Goat anti-human C3 antibodies
Imidazole buffer : ≤ 0.12mol/L
Sodium azide : ≤ %0.1

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[28]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. Sample collection tube with Li heparin should be preferred for plasma.
Lipemic samples are not suitable for testing. Multiple sample freezing and thawing should be avoided.

C3 activity stability in serum and plasma:

4 days at +20/+25 ºC,
8 days at +2/+8ºC,
8 days at -20°C.

Annotation:

- Measurement of C3 can be complicated by the in vitro conversion of C3 to C3c. Due to differences in antibody reactivity against C3 and C3c, C3 concentrations measured in fresh samples may be lower than those determined after long-term storage.[11]

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of a Protein Calibrator .

Protein Calibrator - Lyophilized
Ref.No: VT-012

Calibration stability is 30 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Protein Control Serum I-Lyophilized
Ref.No: VT-013

Protein Control Serum II-Lyophilized
Ref.No: VT-014

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Reference Range [41,42]

Age	Range (mg/dL)
0 day to 3 months	58 – 108
3 months to 6 months	67 – 124
6 months to 9 months	74 – 138
9 months to 12 months	78 – 144
12 months to 10 years	80 – 150
12 years to 18 years	85 – 160
18 years to 30 years	82 – 160
30 years to 40 years	84 – 160
40 years to 70 years	90 – 170

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[29]

Unit Conversion:
mg/dL = 0.01 g/L

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[30]

The determined analytic measuring interval for Complement C3 is 3.7 – 400 mg/dL.

Detection Capability

Limit of Detection (LoD): 2.5 mg/dL.

Limit of Quantitation (LoQ): 3.7 mg/dL.

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[31]

Linearity

This method shows measurement linearity in the activities up to 400 mg/dL.Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[32]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[33]
Repeatability (Within Run) and Repeatability (Day to Day) CV% values of Complement C3 have been given in the table 1 and 2 respectively.

Table 1. Complement C3 Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD*	%CV	n
95 mg/dL	0.94	0.99	80
190 mg/dL	1.21	0.64	80

***SD: Standard Deviation**

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[34]

Table 2. Complement C3 Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD*	%CV	n
95 mg/dL	1.28	1.35	80
190 mg/dL	1.86	1.16	80

***SD: Standard Deviation**

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[34]

Prozone Effect: No prozone effect has been observed up to 600 mg/dL tested for C3.

Interference

Endogenous interferant and analyte concentrations that have been used in the Complement C3 scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[35,36]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Complement C3 interference scanning test is appropriate, is determined as ±10%.[37]

In Complement C3 test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Hemoglobin	: ≤ 250 mg/dL
Bilirubin	: ≤ 20 mg/dL
Rheumatoid Factors	: ≤ 30 IU/mL
Lipemia	: ≤ 2500 mg/dL

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic

antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[36]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

REFERENCES

1. Sahu, A., & Lambris, J. D. (2001). Structure and biology of complement protein C3, a connecting link between innate and acquired immunity. Immunological Reviews, 180, 35–48.
2. Dolmer K, Thirup S, Andersen GR, Sottrup-Jensen L, Nyborg J. Crystallization of human methylamine-treated complement C3 and C3b.
3. Einstein LP, Hansen PJ, Ballow M, et al. Biosynthesis of the third component of complement (C3) in vitro by monocytes from both normal and homozygous C3-deficient humans. J Clin Invest 1977; 60: 963–9.
4. Colten HR, Ooi YM, Edelson PJ. Synthesis and secretion of complement proteins by macrophages. Ann N Y Acad Sci 1979; 332: 482–90.
5. Yu CL, Tsai CY, Hsieh SC, et al. Production of the third component of complement (C3) by peripheral polymorphonuclear neutrophils of the patients with rheumatoid arthritis. Proc Natl Sci Counc Repub China B 1995; 19: 225–32.
6. Kwan WH, van der TouwW, Heeger PS. Complement regulation of T cell immunity. Immunol Res 2012; 54: 247–53.
7. van Kooten C, Fiore N, Trouw LA, et al. Complement production and regulation by dendritic cells: molecular switches between tolerance and immunity. Mol Immunol 2008; 45: 4064–72.
8. Delanghe, J., Speeckaert, R., & Speeckaert, M. M. (2014). Complement C3 and its polymorphism: biological and clinical consequences. Pathology, 46(1), 1–10. https://doi.org/10.1097/pat.0000000000000042
9. Mu¨ller-Eberhard HJ, Schreiber RD. Molecular biology and chemistry of the alternative pathway of complement. Adv Immunol 1980; 29: 1–53.
10. Rodwell, V. W., Bender, D., Botham, K. M., Kennelly, P. J., & Weil, P. A. (2018b). Harper's Illustrated Biochemistry Thirty-First Edition. McGraw Hill Professional.
11. Rifai, N., Chiu, R. W., & Young, I., et al., (2023) Tietz Textbook of Laboratory Medicine (7th ed.), Chapter 31: Amino Acids, Peptides, and Proteins, p.349-349.e42, Elsevier, St. Louis, Missouri 63043
12. Geisbrecht, B. V., Lambris, J. D., & Gros, P. (2022). Complement component C3: A structural perspective and potential therapeutic implications. Seminars in Immunology, 59, 101627. https://doi.org/10.1016/j.smim.2022.101627
13. D. Ricklin, J.D. Lambris, Compement in immune and inflammatory disorders:pathophysiological mechanisms, J. Immunol. 190 (2013) 3831–3838.
14. D. Ricklin, E.S. Reis, J.D. Lambris, Complement in disease: a defence system turning offensive, Nat. Rev. Nephrol. 2016 (12) (2016) 383–401.
15. E.S. Reis, D.C. Mastellos, D. Ricklin, A. Mantovani, J.D. Lambris, Complement in cancer: untangling an intricate relationship, Nat. Rev. Immunol. 18 (2018) 5–18.
16. Ram S, Lewis LA, Rice PA. Infections of people with complement deficiencies and patients who have undergone splenectomy. Clin Microbiol Rev 2010; 23: 740–80.
17. Tichaczek-Goska D. Deficiencies and excessive human complement system activation in disorders of multifarious etiology. Adv Clin Exp Med 2012; 21: 105–14.
18. Sze´plaki G, Proha´szka Z, Duba J, et al. Association of high serum concentration of the third component of complement (C3) with preexisting severe coronary artery disease and new vascular events in women. Atherosclerosis 2004; 177: 383–9.

19. Engstrom G, Hedblad B, Eriksson KF, Janzon L, Lindgarde F. Complement C3 is a risk factor for the development of diabetes: a population based cohort study. Diabetes 2005; 54: 570–5.
20. van Oostrom AJ, Alipour A, Plokker TW, Sniderman AD, Cabezas MC. The metabolic syndrome in relation to complement component 3 and postprandial lipemia in patients from an outpatient lipid clinic and healthy volunteers. Atherosclerosis 2007; 190: 167–73.
21. Wlazlo N, van Greevenbroek MM, Ferreira I, et al. Low-grade inflammation and insulin resistance independently explain substantial parts of the association between body fat and serum C3: the CODAM study. Metabolism 2012; 61: 1787–96.
22. Purwar R, Langer K, Werfel T. Polymorphisms within the C3 gene are associated with specific IgE levels to common allergens and superantigens among atopic dermatitis patients. Exp Dermatol 2009; 18: 30–4.
23. Tietz NW, editor. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders; 1995:164–165.
24. Jacobs DS, DeMott WR, Grady HJ, et al. Laboratory Test Handbook, 4th ed. Hudson, OH: Lexi-Comp; 1996:373–374.
25. Pickering MC,Walport MJ. Links between complement abnormalities and systemic lupus erythematosus. Rheumatology (Oxford) 2000; 39: 133–41.
26. Kim, S. J., Koo, H. M., Lim, B. J., Oh, H. J., Yoo, D. E., Shin, D. H., Lee, M. J., Doh, F. M., Park, S., Yoo, T. H., Kang, S. W., Choi, K. H., Jeong, H. J., & Han, S. H. (2012). Decreased Circulating C3 Levels and Mesangial C3 Deposition Predict Renal Outcome in Patients with IgA Nephropathy. PLOS ONE, 7(7), e40495. https://doi.org/10.1371/journal.pone.0040495
27. Augusto, J., Langs, V., Demiselle, J., Lavigne, C., Brilland, B., Duveau, A., Poli, C., Chevailler, A., Croué, A., Tollis, F., Sayegh, J., & Subra, J. (2016). Low Serum Complement C3 Levels at Diagnosis of Renal ANCA-Associated Vasculitis Is Associated with Poor Prognosis. PLOS ONE, 11(7), e0158871. https://doi.org/10.1371/journal.pone.0158871
28. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI Document EP25-A. Wayne, PA: CLSI; 2009.
29. Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition. CLSI Document EP28-A3c. Wayne, PA: CLSI; 2010.
30. Clinical and Laboratory Standards Institute (CLSI). Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking – 1st Edition. CLSI Document EP34. Wayne, PA: CLSI; 2018.
31. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.
32. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach - 1st Edition. CLSI Document EP06-A. Wayne, PA: CLSI; 2003.
33. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.
34. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.
35. Clinical and Laboratory Standards Institute (CLSI). Supplemental Tables for Interference Testing in Clinical Chemistry - First Edition. CLSI Document EP37. Wayne, PA: CLSI; 2018.
36. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry - Third Edition. CLSI Document EP07. Wayne, PA: CLSI; 2018.
37. CLIA proficiency testing criteria for acceptable analytical performance, as printed in the Federal Register July 11, 2022;87(131:41194-242.
38. Yang, P., Zhu, Z., Zang, Y., Bu, X., Xu, T., Zhong, C., Wang, A., Peng, H., Guo, D., Zheng, X., Xu, T., Chen, J., Zhang, Y., & He, J. (2021). Increased serum complement C3 levels are associated with adverse clinical outcomes after ischemic stroke. *Stroke*, *52*(3), 868–877. https://doi.org/10.1161/strokeaha.120.031715
39. Min, Y. G., Ju, W., Seo, J., Ha, Y., Ban, J., Kwon, Y. N., Jeong, H., Shin, J., Kim, S., Hong, Y., Kim, S. J., & Sung, J. (2023). Serum C3 complement levels predict prognosis and monitor disease activity in Guillain-Barré syndrome. *Journal of the Neurological Sciences*, *444*, 120512. https://doi.org/10.1016/j.jns.2022.120512
40. Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO Publication WHO/DIL/LAB/99.1 Rev. 2: Jan 2002
41. Alper CA, Rose FS. Clinical applications of complement assays. Adv Intern Med. 1975;20:61–88.
42. Whicher J. Complement Component C3. In: Richie RF and Novolotskaia O, eds. Serum Proteins in Clinical Medicine. Scarborough: Foundation of Blood Research; 1996:1–7.

Archem Sağlık Sanayi ve Tic. A.Ş. (With official contract based manufacturing agreement with Validity Sağlık Hiz. Sanayi A.Ş. Company)
Mahmutbey Mah. Halkalı Cad. No:124 Kat:4
Bağcılar/İstanbul/Turkey
Tel: + 90 212 444 08 92
Fax: +90 212 629 98 89
info@archem.com.tr **www.archem.com.tr**
info@validity.com.tr **www.validity.com.tr**

CE

SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests