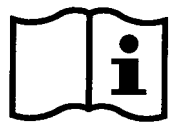


DRG:HYBRID•XL[®]



Instructions for Use

CRP



HYC-5319



80 tests



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Contents / Inhaltsverzeichnis / Contenuti / Contenido / Contenu

1	INTRODUCTION.....	3
2	PRINCIPLE OF THE TEST.....	3
3	WARNINGS AND PRECAUTIONS.....	3
4	REAGENTS.....	3
5	SPECIMEN COLLECTION AND PREPARATION.....	4
6	ASSAY PROCEDURE	4
7	QUALITY CONTROL	4
8	EXPECTED NORMAL VALUES	5
9	LIMITATIONS OF USE	5
10	PERFORMANCE CHARACTERISTICS	5
11	METHOD COMPARISON	5
12	LEGAL ASPECTS	6
13	REFERENCES / LITERATURE	6
	USED SYMBOLS.....	7

1 INTRODUCTION

1.1 Intended Use

The DRG:HYBRiD-XL CRP is an immunoturbidimetric assay for the quantitative in vitro diagnostic measurement of C-reactive protein (CRP) in serum and plasma (heparin and citrate).

Only for use with the DRG:HYBRiD-XL Analyzer.

1.2 Summary and Explanation

Concentration of acute-phase proteins increase in blood as a response to inflammatory disorders (acute-phase response) (1,2). C-reactive protein (CRP), the best known member of this group, is present only at low concentration in blood of healthy individuals (< 5 mg/L), but is elevated up to 500 mg/L in the acute-phase response, caused by inflammatory processes associated with bacterial infections, post-operative conditions or tissue damage. CRP concentration increases already after 6 hours, reaching a peak after 48 hours.

The measurement of CRP represents a useful laboratory test for detection of acute infection as well as for monitoring inflammatory processes in acute rheumatic (3) and gastrointestinal (4) diseases. CRP testing shows various advantages in comparison to the erythrocyte sedimentation rate (ESR) and the leukocyte count. In fact, CRP is more sensitive, shows earlier response and returns to reference range more rapidly after healing.

2 PRINCIPLE OF THE TEST

The DRG:HYBRiD.XL CRP Kit is a immunoturbidimetric assay. It is based on an endpoint determination of the CRP concentration by photometric measurement of the antigen-antibody reaction between antibodies against human CRP and CRP that is present in the sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. This kit can only be used in combination with the DRG:HYBRiD-XL Analyzer
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is clear and understood.
4. Do not remove, exchange, discard or damage any of the barcode labels provided with each kit and its components. All barcodes build an integral system for the kit lot.
5. Respect the general safety measures for use of laboratory reagents.
6. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
7. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
9. Wear appropriate disposable gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may cause false results.
10. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
11. Do not use reagents beyond expiry date as shown on the kit labels.
12. Unused reagent cartridges must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant provided.
13. Optimal test results are only obtained when using calibrated pipettes.
14. Do not mix or use components from kits with different lot numbers. It is advised not to interchange reagent cartridges of different kits even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the wells in the reagent cartridges may differ slightly.
15. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
16. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.

17. For information on hazardous substances included in the kit please refer to Safety Data Sheets.
For professional users the Safety Data Sheet for this product is available upon request directly from DRG.

4 REAGENTS

4.1 Reagents provided

4.1.1 Reagent Cartridges

80 pieces containing the following:

- **Reagent 1**, 260 µL
TRIS pH 7.5, 100 mmol/L, Polyethylenglycol (PEG), detergents and stabilizers.
- **Reagent 2**, 100 µL
TRIS pH 8.0, 100 mmol/L, Anti-human CRP antibodies (goat) with stabilizers.

4.1.2 Re-Calibrator 1 & 2

2 vials, 0.5 mL each, ready to use;

For re-calibration of the quantitative DRG:HYBRiD-XL CRP test.

Concentrations are lot-specific.

Contain non-mercury preservative.

4.1.3 Control 1 & 2

2 vials, 1.0 mL each, ready to use;

For control values and ranges please refer to the bar code on vial label or to the QC-Datasheet.

See "Reagent Preparation".

Contain non-mercury preservative.

4.2 Materials required but not provided

- General needed laboratory equipment
- Ultra-pure water
DRG recommends to use Clinical Laboratory Reagent Water (CLRW) according to CLSI guideline 3C-A4 with the following specifications:
Resistivity at 25 °C [MΩ·cm]: > 10
Conductivity at 25 °C [µS·cm⁻¹]: < 0.1
Total Organic Carbon/p.p.b.(µg/L) : < 50
Colloids [µg/mL]: <0.05
- **REF** HYB-5252 DRG:HYBRiD-XL Analyzer
- **REF** HYI-5392: *System Solution 5L*, 5000 mL;
(Instrument Feed Water according to CLSI guideline 3C-A4 with the following specification can also be used:
Resistivity at 25 °C [MΩ·cm]: > 1
Conductivity at 25 °C [µS·cm⁻¹]: < 1
Total Organic Carbon/p.p.b.(µg/L) : < 200
Colloids [µg/mL]: <0.1)
- **REF** HYI-5394: *Wash Buffer*, 40x concentrate, 25 mL
- **REF** HYI-5395: *Needle Cleaning Solution*, 30 mL. Cleaning solution for the pipetting needle (daily and weekly maintenance, see also user manual)
- **REF** HYI-5387: *Cuvettes*, (2 x 360 pieces)
- NaCl solution 9 g/L, (for sample dilution, if necessary)

For use of the *Secondary Sample Holder* for secondary tubes the following tubes are required:

- **REF** HYI-5391: *Sample Tubes (Secondary)*, 2500 pcs.

4.3 Storage Conditions

When stored at 2 °C to 8 °C, unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents and the reagent cartridges must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to tightly close it again.

Opened kits retain activity for two months if stored as described above.

4.4 Reagent Preparation

Bring all reagents, such as controls and re-calibrators, to room temperature (20 °C to 25 °C) prior to use. Reagent Cartridges can be used directly after storage in the refrigerator.

Wash Buffer (not included in the kit)

For Wash Buffer (1x) dilute 25 mL of Wash Buffer (40x) with 975 mL ultra-pure water to a final volume of 1000 mL.

The diluted Wash Buffer (1x) is stable for 2 weeks at room temperature.

4.5 Disposal of the Kit

The disposal of the kit and all used materials/reagents must be performed according to the national regulations. Special information for this product is given in the Safety Data Sheet.

4.6 Damaged Test Kits

In case of any damage to the test kit or components, DRG must be informed in writing, at the latest one week after receiving the kit. Damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed of according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (Heparin- or citrate plasma) can be used in this assay.

Attention:

- This test was not verified with blood collection tubes of all available manufacturers.
- Sample Collection Systems of some manufacturers may contain different materials which in isolated cases could affect the test results.
- If primary tubes for sample collection are used, please follow the instructions of the manufacturer.
- Do not use haemolytic, icteric or lipaemic specimens.
- Samples containing precipitates have to be centrifuged prior to the test run.
- Do not use heat inactivated samples.
- Do not use standards or external controls stabilized with azide.

5.1 Specimen Collection**Serum:**

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 1 day at 2 °C to 8 °C prior to performing the assay.

Specimens stored for a longer time (up to two months) should be frozen only once at -20 °C prior to the assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution**5.3.1 Manual Sample Dilution**

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with 0.9% NaCl solution and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

- a) dilution 1:10: 10 µL sample + 90 µL 0.9% NaCl solution (mix thoroughly)
- b) dilution 1:100: 10 µL dilution a) 1:10 + 0.9% NaCl solution (mix thoroughly).

6 ASSAY PROCEDURE**6.1 General Remarks**

- All reagents, such as controls and re-calibrators and specimens, must be allowed to come to room temperature (20 °C to 25 °C) before use.
All reagents and samples must be mixed without foaming. Reagent Cartridges can be used directly after storage in the refrigerator.
- Once the test has been started, all steps should be completed without interruption.

- Samples, controls and re-calibrators should be measured within 2 hours in order to avoid possible evaporation effects.
- The *Secondary Sample Holder* (HYI-5437) for secondary tubes has the capacity for a maximum of 20 samples including controls and re-calibrators. They all have to be pipetted into the secondary tubes, and the respective barcodes of control/re-calibrator vials and, if available, the sample barcodes have to be read with the external barcode scanner.

6.2 Test Procedure

- To ensure proper operation of the test, the instructions in the user manual for the DRG:HYBRiD-XL should strictly be followed.
- All test specific information required for the correct operation is included in the respective barcodes of the reagents.
Take care not to damage these bar codes!
- **It is recommended to tap the bottom of the Cartridge Segments containing the reagent cartridges once on the bench before placing them on the rotor. This is to avoid foam and adhering of the liquid on the sealing of the reagent cartridge.**
- Place reagent cartridges on the rotor of the unit. The heating to 37 °C incubation temperature is performed automatically in the unit.

6.3 Calibration**Traceability:**

This method was standardized against the DRG CRP ELISA (EIA-1952).

Each DRG:HYBRiD-XL reagent contains a barcode with the specific information for re-calibration of the reagent lot. The Master Curve is printed as a 2-D barcode on the outer label of the kit package and on the QC-Datasheet and has to be scanned with the external barcode scanner prior to the first use of the respective kit lot.

Re-calibration is recommended:

- if one or both assay controls are found outside the specified range.
- after 4 weeks of use of the same reagent kit on the unit.

6.4 Calculation of Results

The analyte concentrations are calculated automatically by the DRG:HYBRiD-XL's system software.

7 QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

It is also recommended to participate in national or international Quality Assessment programs in order to ensure the accuracy of the results.

Apply appropriate statistical methods for analysing control values and trends. If the results of the assay do not agree with the established acceptable ranges of control materials, patient results should be considered invalid. In this case, please check the following: expiration dates and storage conditions of reagents, operational reliability of the analyser. In addition, it is indicated to perform a Re-calibration.

In case of further questions please contact your local distributor or DRG directly.

7.1 Internal Controls

For Quality Control it is necessary to use the two internal controls provided with each kit.

Acceptance ranges for both internal controls (Control 1 & 2) were established by the manufacturer and are summarized in the QC-Datasheet added to the kit. Note that the expected values and acceptance ranges stated in the QC-Datasheet always refer to the current kit lot.

Internal controls should be run in single determination:

- on a routine basis (e.g. once per 24 h)
- if re-calibration is required (if one or both internal controls are out of range)
- if a new kit lot is used (in order to avoid any negative impact on the kit performance by improper transport or to detect improper storage during transport).

7.2 External Controls

Use controls at both normal and pathological levels.

The control intervals and control ranges for external controls should be adapted to the individual requirements of each laboratory. All results must be within the defined limits.

Each laboratory should establish corrective measures to be taken if values of external controls are not found in the acceptance range.

8 EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and pathological values.

Normal concentration in healthy human serum is usually lower than 5 mg/L, slightly increasing with aging.

Higher levels are found in late pregnant women, mild inflammation and viral infections (10 - 40 mg/L), active inflammation, bacterial infection (40 - 200 mg/L), severe bacterial infections and burns (> 200 mg/L).^[5]

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated with other clinical observations and diagnostic tests.

9 LIMITATIONS OF USE

Reliable and reproducible results will be obtained, when the assay procedure is performed with a complete understanding of the instructions for use and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

10 PERFORMANCE CHARACTERISTICS

10.1 Assay Dynamic Range

The dynamic range of the assay is defined by the limit of blank and the maximum value of the Master Curve.

Values below the measuring range are indicated as "< 1.0 mg/L". Values above the measuring range are indicated as "> 250 mg/L".

The range of the assay is between 1.0 mg/L – 250 mg/L.

10.2 Specificity of Antibodies (Cross-Reactivity)

Due to its antibodies, the DRG CRP Test is a specific immunoassay for human CRP.

10.3 Sensitivity

The sensitivity study was designed according to CLSI guideline EP17-A2.

The Limit of Blank (LoB) is 1.0 mg/L.

The Limit of Detection (LoD) is 2.5 mg/L.

The Limit of Quantification (LoQ) is 7.0 mg/L.

10.4 Precision Performance

The precision study was designed according to CLSI guideline EP5-A2.

10.4.1 Within-Run (Intra Assay)

Samples were analysed 2 times daily in duplicates for 20 days (n=80). CV (%) is given as mean of 40 duplicate CVs. The within-run variability is shown below:

Sample	n	Mean (mg/L)	CV (%)
1	40	65.1	2.9
2	40	19.2	6.7
3	40	8.8	5.0
4	40	8.9	6.4

10.4.2 Total Precision (Inter Assay)

Samples were analysed 2 times daily in duplicates for 20 days (n=80). The total precision is shown below:

Sample	n	Mean (mg/L)	CV (%)
1	80	65.1	7.1
2	80	25.5	9.1
3	80	19.2	12.1
4	80	8.9	13.7

10.4.3 Inter-Lot

The between-lots variation was determined by 6 measurements of 4 samples with 3 different kit lots.

Sample	n	Mean (mg/L)	CV (%)
1	18	66.3	8.1
2	18	60.3	7.5
3	18	39.6	7.7
4	18	9.7	12.4

10.5 Recovery

Recovery was determined by adding four increasing concentrations of the analyte to four different patient samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was assayed and analyte concentrations of the samples were calculated from the Master Curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

Sample	1	2	3	4
Concentration (mg/L)	8.21	27.2	33.0	69.8
Average Recovery (%)	98.0	97.1	94.6	96.2
Range of Recovery (%)	from 94.2 to 101.0	93.4 102.2	88.3 96.3	90.5 100.6

10.6 Linearity

Four samples containing different amounts of analyte were serially diluted with 0.9% NaCl solution. The percentage recovery was calculated by comparing the expected and measured values for the analyte.

Sample	1	2	3	4
Concentration (mg/L)	194.0	242.5	497.0	1243.8
Average Recovery (%)	102.9	101.5	111.7	97.7
Range of Recovery (%)	from 96.1 to 114.8	85.6 114.9	106.3 114.9	91.8 105.6

10.7 Interfering Substances

Haemoglobin (up to 1 mg/mL), Bilirubin (up to 0.12 mg/mL) and Triglyceride (up to 7.5 mg/mL) have no influence on the assay results.

Until today no substances (drugs) are known to us, which have an influence to the measurement of CRP in a sample.

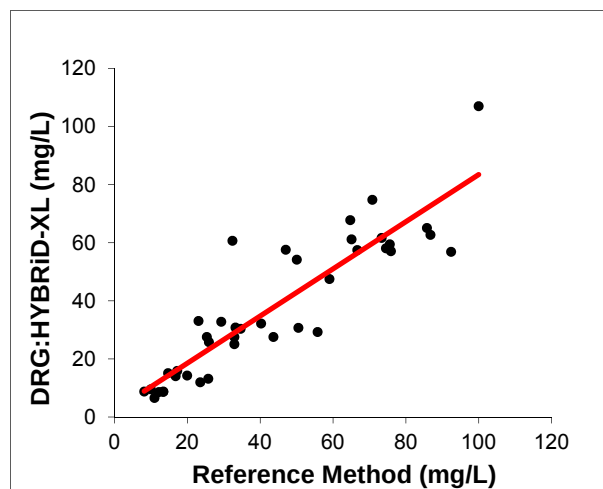
10.8 High-Dose-Hook Effect

No hook effect was observed in this test up to 1000 mg/L of CRP.

11 METHOD COMPARISON

A comparison of DRG:HYBRiD-XL ELISA CRP Test HYC-5319 (y) and DRG ELISA EIA-1952 (x) using clinical samples gave the following correlation:

$$\begin{aligned} n &= 40 \\ r &= 0.900 \\ y &= 0.8121x + 2.2501 \end{aligned}$$



12 LEGAL ASPECTS

12.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

12.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 12.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

12.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different kit lots could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 12.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

For further information please refer to the User Manual of the DRG:HYBRiD-XL, analyser-specific application sheets, product information and package inserts of all necessary components.

13 REFERENCES / LITERATURE

1. Agrwal A, Kilpatrick JM, Volanakis JE. Structure and function of human C-reactive protein. In: Mackiewicz A, Kushner I, Baumann H. eds. Acute phase proteins. London: CRC Press, 1993, 79-92.
2. Whicher JT, Rifai N, Biasucci LM. Markers of the acute phase response in cardiovascular disease: an update. Clin Chem Lab Med 2001, 39, 85-88.
3. Thomson D, Whicher JT, Banks RE. Acute phase reactions in predicting disease outcome. In Emery P, ed. Management of early inflammatory arthritis. Balliere's Clinical Rheumatology. London; Balliere Tyn dall, 1992, 393-404.
4. Chalmers RE, Stross P, Barry RE, Whicher JT. Serum amyloid A protein compared with C-reactive protein, alpha 1-anti-chymotrypsin and alpha 1-acid-glycoprotein a monitor of inflammatory bowel disease. Eur J Clin Invest. 1987, 17, 460-467.
5. Clyne B, Olshaker Js. The C-Reactive Protein. The Journal of Emergency Medicine. 1999, 17, 1019-1025.

USED SYMBOLS

Symbol	English	Deutsch	Italiano	Español	Français
	European Conformity	CE-Konformitätskennzeichnung	Conformità europea	Conformidad europea	Conforme aux normes européennes
	Consult instructions for use*	Gebrauchsanweisung beachten*	Consultare le istruzioni per l'uso	Consulte las Instrucciones	Consulter les instructions d'utilisation
IVD	In vitro diagnostic medical device*	In-vitro-Diagnostik*	Per uso Diagnostica in vitro	Para uso Diagnóstico in vitro	Usage Diagnostic in vitro
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
REF	Catalogue number*	Bestellnummer*	No. di Cat.	Número de catálogo	Référence
LOT	Batch code*	Chargenbezeichnung*	Lotto no	Número de lote	No. de lot
	Sufficient for *	Ausreichend für *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature Limitation*	Temperaturbegrenzung*	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use by*	Verwendbar bis*	Data di scadenza	Fecha de caducidad	Date limite d'utilisation
	Manufacturer*	Hersteller*	Fabbricante	Fabricante	Fabricant
	Caution*	Achtung*			
<i>Distributed by</i>	Distributor	Vertreiber	Distributore	Distribuidor	Distributeur
<i>Content</i>	Content	Inhalt	Contenuto	Contenido	Contenu
<i>Volume/No.</i>	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Numéro
<i>Reagent Cartridge</i>	Reagent Cartridge				
<i>Re-Calibrator</i>	Re-Calibrator	Re-Kalibrator			
<i>Control</i>	Control	Kontrolle	Controllo	Control	Contrôle
<i>Enzyme Conjugate</i>	Enzyme Conjugate	Enzymkonjugat	Tracciante enzimatico	Conjugado enzimático	Conjugué enzymatique
<i>Substrate Solution</i>	Substrate Solution	Substratlösung	Soluzione di substrato	Solución de sustrato	Solution substrat
<i>Sample Diluent</i>	Sample Diluent	Probenverdünnungsmedium	Diluente dei campioni	Solución para dilución de la muestra	Solution pour dilution de l'échantillon
<i>Wash Buffer</i>	Wash Buffer	Waschpuffer			
<i>System Solution</i>	System Solution	Systemlösung			
<i>Needle Cleaning Solution</i>	Needle Cleaning Solution	Nadel-Reinigungslösung			