



Application Note

Guideline for Determination of Retinol-Binding Protein in Serum on Hitachi 917

General information

Intended use	The Application Note is intended for the quantitative determination of Retinol-Binding Protein in human sample material by turbidimetry on Hitachi 917 (1, 2).																					
Measuring range	Approximately 10.0-130.0 mg/L depending on the specific lot of the calibrator. In case of post-dilution the range can be expanded to 10-208 mg/L.																					
Reference interval	26 - 60 mg/L. It is recommended to determine the reference interval for the local population.																					
Instrument settings	Instrument programming is performed according to "Instrument Settings" on page 3.																					
Reagents	<table border="0"> <thead> <tr> <th></th> <th style="text-align: left;">Code</th> <th style="text-align: left;">Name</th> </tr> </thead> <tbody> <tr> <td><u>Antibody</u></td> <td>A0040</td> <td>Dako Polyclonal Rabbit Anti-Human Retinol-Binding Protein</td> </tr> <tr> <td><u>Reaction buffer</u></td> <td>S2007</td> <td>Dako Reaction buffer 1</td> </tr> <tr> <td><u>Diluent</u></td> <td>S2005</td> <td>Dako Dilution Buffer 1</td> </tr> <tr> <td><u>Calibrator</u></td> <td>X0908</td> <td>Dako Human Serum Protein Calibrator</td> </tr> <tr> <td><u>Controls</u></td> <td>X0939</td> <td>Dako Human Serum Protein Low Control</td> </tr> <tr> <td></td> <td>X0940</td> <td>Dako Human Serum Protein High Control</td> </tr> </tbody> </table>		Code	Name	<u>Antibody</u>	A0040	Dako Polyclonal Rabbit Anti-Human Retinol-Binding Protein	<u>Reaction buffer</u>	S2007	Dako Reaction buffer 1	<u>Diluent</u>	S2005	Dako Dilution Buffer 1	<u>Calibrator</u>	X0908	Dako Human Serum Protein Calibrator	<u>Controls</u>	X0939	Dako Human Serum Protein Low Control		X0940	Dako Human Serum Protein High Control
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Samples	Human serum. Stable for 7 days at 2-8 °C. Stable for 3 months at -20 °C (if frozen only once). Frozen samples should be thawed at 37 °C and mixed well before analysis.																					
Calibrator	Dilution of standards is performed automatically by the instrument.																					
Reaction buffer (R1)	The reaction buffer is ready to use. On board stability is 28 days.																					
Antibody (R3)	<p>Predilute the antibody 1:1.67 (e.g. 2000 µL antibody + 1340 µL diluent). If in rare cases the prediluted antibody appears slightly turbid, filtration through a 0.22 µm membrane filter is recommended. Stability of undiluted antibody: See expiry on the label. Stability of prediluted antibody: 28 days at 2-8 °C. On board stability: 28 days.</p> <p><u>Capacity:</u> 1 mL of prediluted antibody is equivalent to approximately 18 cuvette readings of standards or samples. The dead volume of the reagent bottle should be considered when calculating the required amount of reagent.</p>																					
Calibration stability	It is recommended to recalibrate every 28th day or when reagent lots change, a new antibody dilution is prepared, the antibody dilution is filtered, or quality control results fall outside the range as established by the individual laboratory.																					
Trouble shooting	If performance is unacceptable, try to recalibrate. Check reagents and procedure. If the problem persists, please contact instrument supplier or Dako Technical Service.																					

Performance Data

Sensitivity An OD value of approximately 0.230 on Hitachi 917 corresponds to a concentration around 130 mg/L Retinol -Binding Protein.

Detection limit The detection limit is estimated to 1.3 mg/L.

Precision The precision was estimated by testing at 4 different Retinol-Binding Protein (RBP) levels by ANOVA analysis of 6 runs each with a new calibration and 6 determinations in each run.

Sera	RBP Mean value (mg/L)	Standard deviation (mg/L)			Total CV (%)	n
		Within run	Between run	Total		
1	34.2	0.330	0.402	0.520	1.5	36
2	87.1	0.739	1.934	2.071	2.4	36
3	20.5	0.267	0.764	0.810	4.0	36
4	115.9	0.882	3.149	3.270	2.8	36

Accuracy A recovery of Retinol-Binding Protein of 90-110% can be expected for Dako Human Serum Protein Low Control, Code X0939, and Dako Human Serum Protein High Control, Code X0940.

Linearity The assay is linear in the measuring range.

Security range No antigen excess is found for Retinol-Binding Protein concentrations up to 553.5 mg/L.

Interference No interference is found at concentrations up to 5 g/L of hemoglobin, 300 mg/L of bilirubin, 50 U/mL of Rheumafactor and 10 g/L of triglyceride.

- References**
1. Blirup-Jensen S. Protein Standardization III: Method optimization: Basic principles for quantitative determination of human serum proteins on automated instruments based on turbidimetry or nephelometry. Clin Chem Lab Med 2001;39:1098-1109.
 2. Hitachi 917 manual(s).

Instrument Settings (Software Version 7146020-02-48)

Analyze

Assay/Time/Point	2Point End	10	15 34 0 0			
Wave (2nd/Primary)	700	340				
S.Vol (Normal)	8	0	0			
S.Vol (Decrease)	5	0	0			
S.Vol (Increase)	0	0	0			
Diluent	951*					
Reagent (R1) T1	250	0	901*	0		
Reagent (R2) T2	0	0	901*	0		
Reagent (R3) T3	50	20	901*	0		
Reagent (R4) T4	0	0	901*	0		
Abs. Limit	32000	Increase	2 Tests			
Prozone Limit	32000	34	Upper			
Cell Detergent	Detergent 1					

Calib

Calibration Type	Logit-Log 4P					
Point	6	Span Point	4			
Weight	0					
Auto Calibration						
Blank	0	Lot				
Span	0	Bottle				
2 Point	0					
Full	0					
SD Limit	50					
Duplicate Limit	20	%	100	Abs		
Sensitivity Limit	-99999	99999				
S1 ABS Limit	-32000	32000				

Range

Application Code	901*	Unit	mg/L
Report Name	RBP		
Data Mode	On Board		
Control Interval	*		
Instrument Factor (Y=ax+b)	a= 1.0	b= 0	
Technical Limit**	st1+0.001	st 6	
Repeat Limit*	st 1	st 6	
Expected Value			
(Male)	100 Y	-99999	99999
	100 Y	-99999	99999
		-99999	99999
(Female)	100 Y	-99999	99999
	100 Y	-99999	99999
		-99999	99999
(Default)	Male	Range 1	
Qualitative	Cancel		
(1)	0	..	
(2)	0	-	
(3)	0	+	
(4)	0	++	
(5)	0	+++	
(6)		++++	

Others

<Standard>	(1)	(2)	(3)	(4)	(5)	(6)
Calibration Code*	003	003	003	003	003	003
Concentration***	0.2500	0.3125	0.5000	0.9000	1.500	3.000
Position*	X C _(cal)	X C _(cal)	X C _(cal)	X C _(cal)	X C _(cal)	X C _(cal)
Sample Volume	35	35	4	7.2	12	24
Diluted S. Vol	8.0	10.0	0.0	0.0	0.0	0.0
Diluted Volume	105	105	0	0	0	0

Diluent – Code S2005

R1 – Code S2007

R3 – Code A0040, diluted 1:1.67.

[*] Defined by the customer.

[**] The calculated relative concentration of Std.1 and Std.6. In order to get a warning for samples with concentration below the concentration of the lowest standard, the lower technical limit has to be 0.001 mg/L higher than the lowest standard.

[***] Calibrator, Code X0908. The concentration is calculated as the factor times the calibrator value for the specific lot (stated in g/L on the X0908 Analytical Value Sheet). Number of decimals stated for Std.1 defines the number of decimals in printout. One decimal is recommended.