Comparison of various VACUETTE® Glucose Tubes to the Vacutainer® Glucose Tube

Background:

Greiner Bio-One offers various blood collection tubes for determination of glucose concentration.

These tubes are available with different additives and are suitable for the analysis of glucose concentration within 48h [1]:

- VACUETTE® FX Sodium Fluoride/Potassium Oxalate
- VACUETTE® FE Sodium Fluoride/K_3EDTA

Sodium fluoride is used in VACUETTE® Glucose Tubes as a glycolytic inhibitor to preserve glucose and is combined with an anticoagulant such as EDTA or potassium oxalate [2]. This additive composition inhibits the enolase in the glycolysis pathway providing the long-term stabilization of glucose concentration, with the exception of the initial 2-4 hours after blood collection when the enzymes hexokinase and phosphofructokinase are active in this biochemical pathway.

The additive compositions meet the requirements provided in ISO 6710 [3].

Study Objective:

A clinical study, including 20 male and female subjects aged 18-64, was carried out to compare the performance of various VACUETTE® standard glucose tubes in comparison to a VACUTAINER® glucose tube to demonstrate the stability of glucose concentration in aliquoted plasma samples.

Study design:

The following tube types were used in this study:

Sample ID	Description	Tube dimension	Prod. No.	Volume
A	VACUTAINER® Fluoride/Oxalate	13/75	368920	2 ml
B	VACUETTE® FX Sodium Fluoride/Potassium Oxalate	13/75	454061	2 ml
C	VACUETTE® FE Sodium Fluoride/K_3EDTA	13/75	454085	2 ml

The study has been approved by Ethics Commission. Informed consent was given by all participants.

Blood was drawn from 20 donors aged 18-64 into 1 tube of each type using an SBC-Set (#450085), a tourniquet (#840050) and a discard tube. All tubes were gently inverted 8x after collection to mix the blood with the additive. All samples A were centrifuged at 1300g for 10 min at 20°C and all samples B and C were centrifuged at 1800g for 10 min at 20°C in a swing bucket centrifuge (Hettich Rotanta 460R). After centrifugation, the plasma was transferred into a secondary tube.

Analysis of glucose concentration was done at the following time points:

- Initial (t_0), 24h ±1 (t_{24}), 36h ±1 (t_{36}), and 48h ±1 (t_{48})

All tubes were stored at room temperature between the time points for analysis.

Glucose concentration and Hemolysis index (LIH) were measured immediately after centrifugation for the initial time point and all subsequent replicate analyses using an AU680 instrument from Beckman Coulter (serial no. 2012101804). Analysis was performed with the instrument's accompanying reagents.

Statistical analysis was done by STATISTICA 13.2 and clinical assessment was performed on basis of the acceptance criteria given for glucose (11%) and LIH (<500 mg/dl free hemoglobin) in RiliBaek [4].

Results:

Comparison of sample A (BD Vacutainer®) to B1 – C1 (GBO VACUETTE®) measured at t_{0h} (initial analysis out of plasma): The detailed statistics are given in the annex by way of a bar plot, correlation results, difference plot and Bland Altman analysis.

No clinically significant deviations in glucose concentrations were found comparing Vacutainer® Fluoride/Oxalate tubes and the VACUETTE® Glucose tubes (VACUETTE® FX Sodium Fluoride/Potassium Oxalate tubes and VACUETTE® FE Sodium Fluoride/K_3EDTA tubes) at initial time point when analyzed using the plasma transferred into a secondary tube immediately after centrifugation.

The initial analysis of HIL resulted in free hemoglobin values < 100 mg/dl.

Stability of glucose concentration in all tested glucose tubes – analysis using plasma stored following transfer into a secondary tube:

Sample	**Glucose concentration mean values ± SD in mg/dl**			
	Initial plasma aliquot	Plasma after 24h in a secondary tube	Plasma after 36h in a secondary tube	Plasma after 48h in a secondary tube
A	93.80±15.42	93.70±15.39	92.80±15.35	93.90±15.39
B	93.75±14.93	93.80±15.01	93.10±14.93	94.05±14.93
C	95.45±14.83	95.20±14.57	94.40±14.40	95.32±15.15

The table above shows the mean values of glucose concentration for initial analysis of plasma aliquots and the replicate analyses after storage in a secondary tube at room temperature.

None of the tested samples (Vacutainer® Fluoride/Oxalate, VACUETTE® FX Sodium Fluoride/Potassium Oxalate, VACUETTE® FE Sodium Fluoride/K_3EDTA) had clinically or analytically significant deviations in glucose concentration comparing the time points t_{24}, t_{36} and t_{48} to the initial time point t_0 when analyzed using plasma transferred to secondary tubes.

The differences between the time points t_{24}, t_{36} and t_{48} to the initial time point t_0 for all tubes are within the acceptance criteria of 11% recommended by the guideline RiliBaek [4].

Glucose concentration in all tested glucose tubes when analyzed using plasma from the primary tube after whole blood storage compared to the initial plasma glucose concentration:

The following table indicates the analytical results for glucose concentration in all tubes at the time points t_{24h}, t_{36h} and t_{48h} compared to the initial plasma glucose concentration when tested using plasma from the primary tube after whole blood storage.

Sample	**Glucose concentration mean values ± SD in mg/dl**			
	Initial plasma aliquot	After 24h whole blood storage	After 36h whole blood storage	After 48h whole blood storage
A	93.80±15.42	88.05±15.05*	88.60±13.22	89.70±12.87
B	93.75±14.93	88.40±15.12*	89.05±13.30*	89.55±12.72*
C	95.45±14.83	88.30±14.44*	88.70±13.12*	88.80±12.40*

*higher difference to initial plasma aliquot than recommended by RiliBaek [4] when compared to initial value

Due to the uninhibited glycolysis in blood collection tubes that do not contain an additive such as citrate, which acts as a short-term inhibitor of glycolysis by inactivating the enzymes hexokinase and phosphofructokinase, glucose concentration declines in the first hours following blood collection if plasma is not separated into a secondary tube immediately after centrifugation. After the initial drop, the glucose concentration remains stable up to 48h with whole blood storage. The resulting deviations with whole blood storage in comparison to the initially aliquoted plasma are slightly higher than the 11% deviation recommended by the RiliBaek guideline.[4]

Hemolysis in the Glucose Tubes Tested

According to literature, the cell membrane damage caused by fluoride results in a higher hemolysis rate in sodium fluoride/potassium oxalate tubes than in tubes without fluoride [5].

The results of this study confirm literature findings with demonstrated hemolysis in tubes (samples A-C) after long term storage.

The analysis of HIL at all replicate time points resulted in free hemoglobin values < 100 mg/dl.

Conclusion

Equivalent performance was demonstrated for all tested VACUETTE® Standard Glucose tubes (Samples B – C) and Vacutainer® Fluoride/Oxalate tubes (Sample A) for the initial analysis of glucose concentration at t_{0h}.

Equivalent performance was demonstrated for all tested VACUETTE® Glucose tubes (Samples B – C) and Vacutainer® Fluoride/Oxalate tubes (Sample A) for the time period of 48 hours when measured using aliquoted plasma stored at room temperature.

In view of stability, deviations higher than the acceptance criteria of 11% were found in all samples when stored as whole blood for 24, 36 or 48h at room temperature due to lack of specific additive to inhibit the glycolysis enzymes immediately following collection.

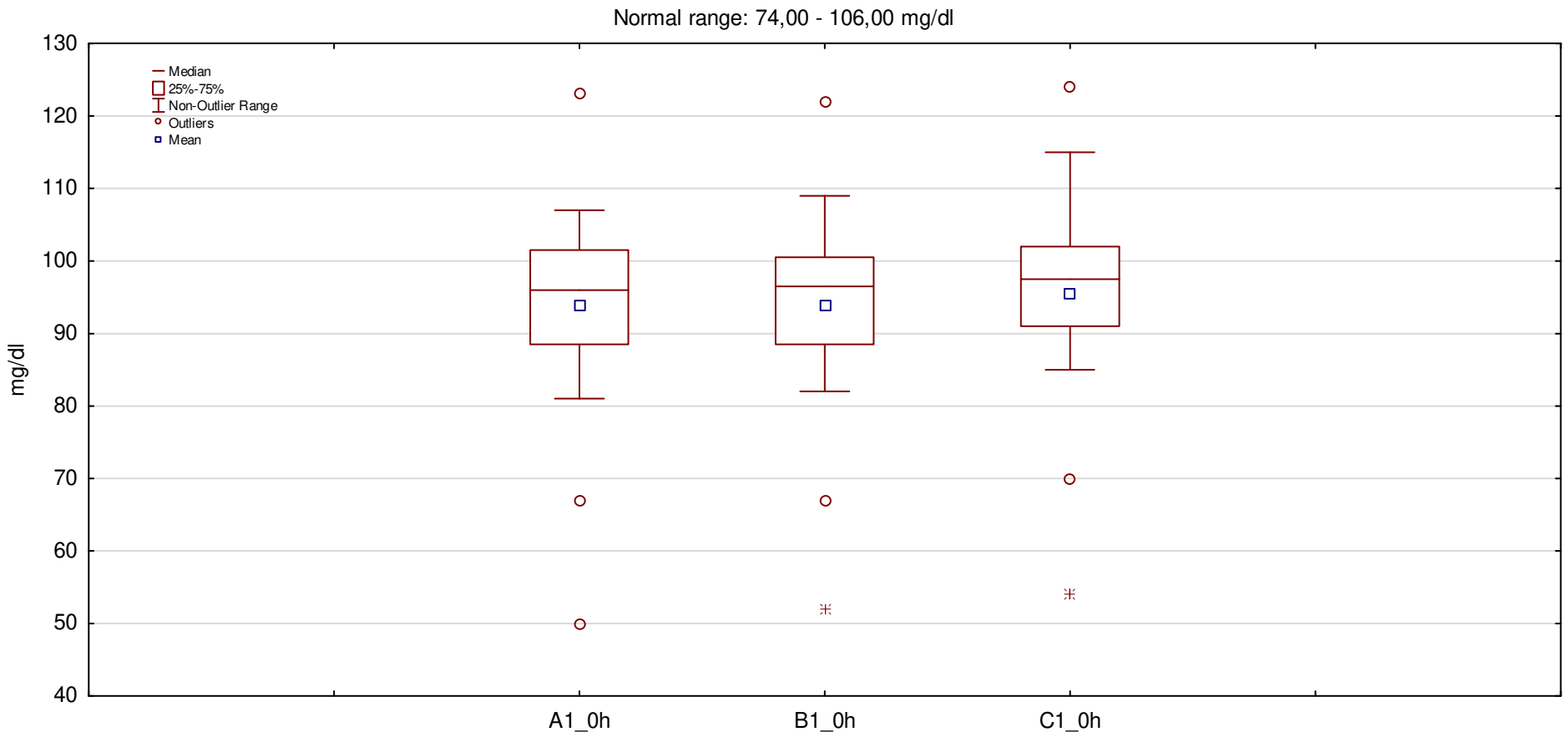
References:

[1] Greiner Bio-One. Evacuated Blood Collection System 980200_Rev.19. Instructions for Use. Kremsmünster, Austria. 2017.
[2] Greiner Bio-One. Product Information G01_E-Rev02
[3] ISO 6710:1995(E), Single-use containers for venous blood specimen collection. International Standard. 1995
[4] RILIBAEK: Guideline of the German Medical Association for Quality Assurance.
[5] Lippi, G., Nybo, M., Cadamuro, J., Guimaraes, J. T., van Dongen-Lases, E., & Simundic, A. M. (2018). Blood Glucose Determination: Effect of Tube Additives. Advances in Clinical Chemistry, 84, 101–123.

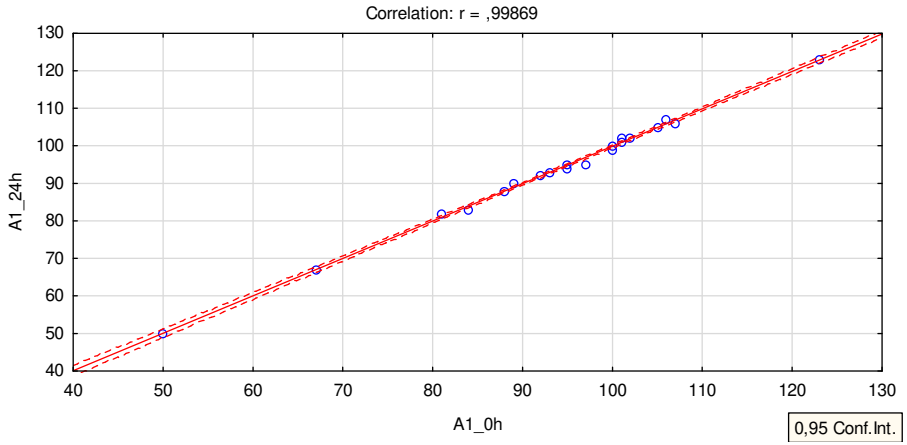
Annex:

Glucose (Gluc)

Normal range: 74 - 106 mg/dl



Correlation: Gluc [mg/dl] A1_0h/A1_24h



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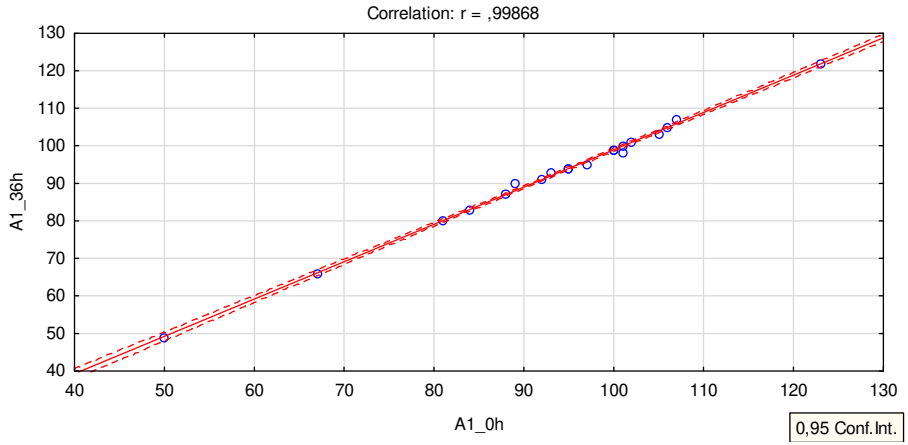

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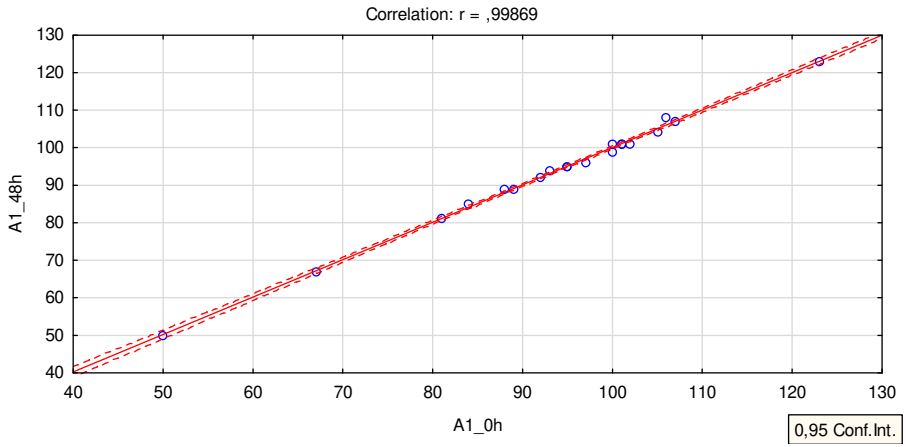

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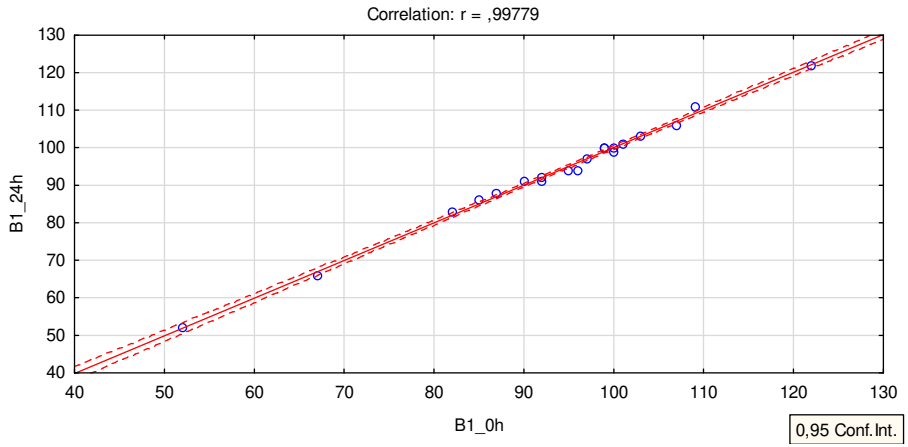

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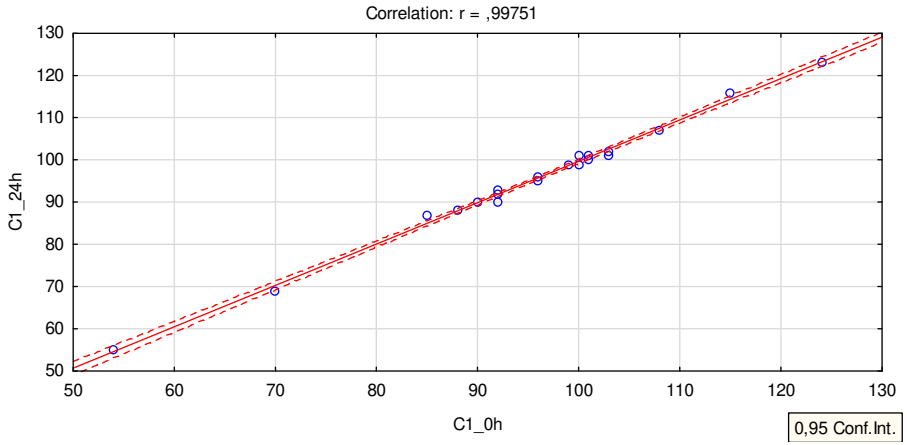

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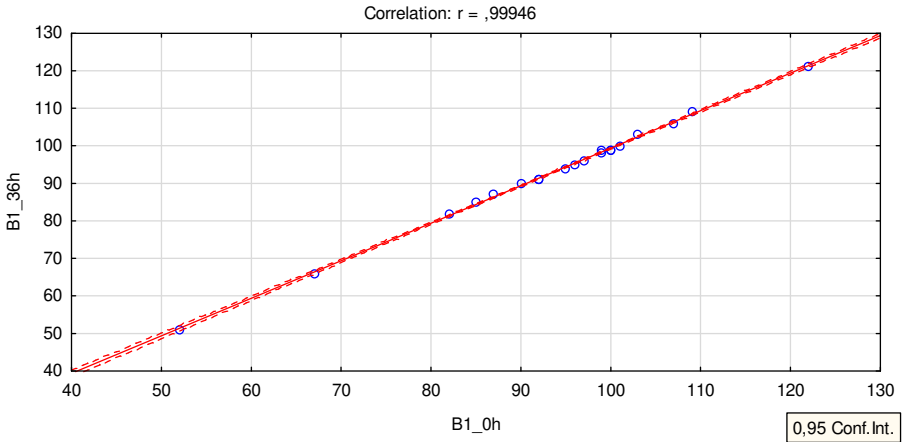

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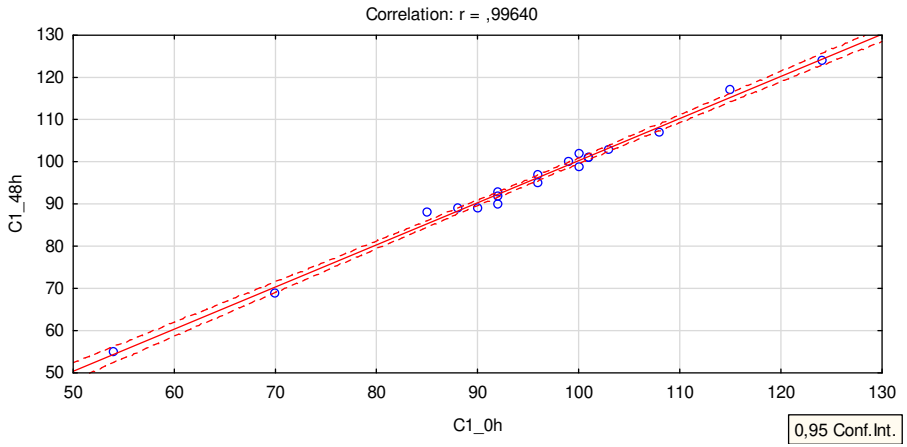

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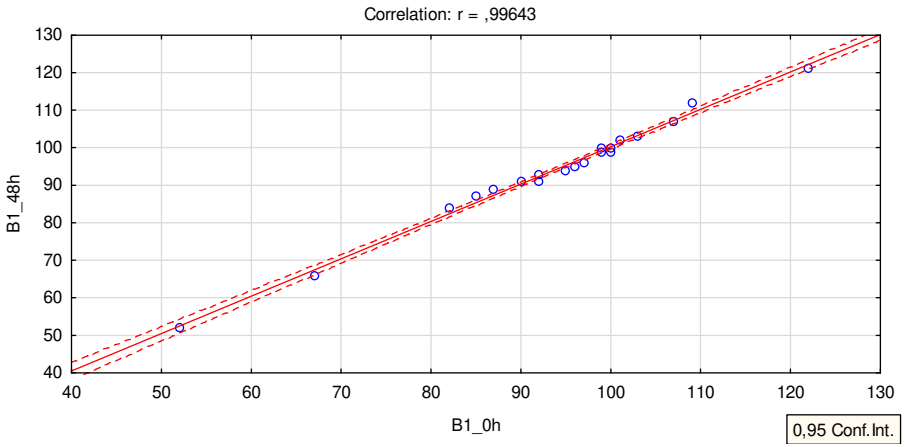

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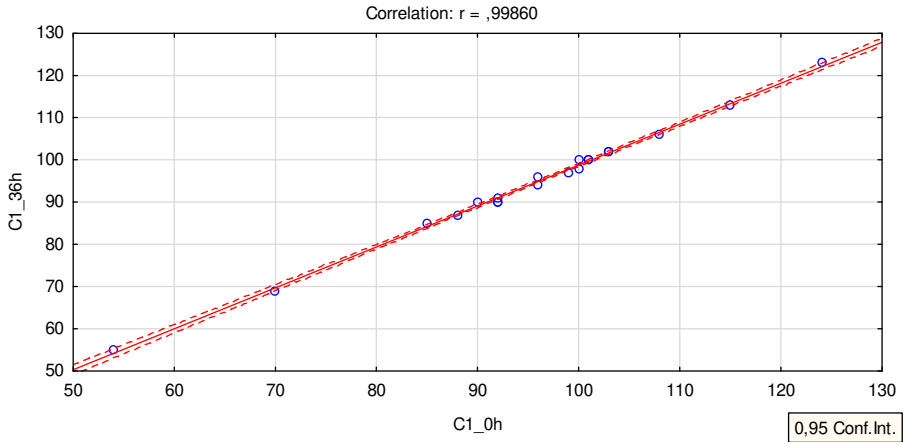
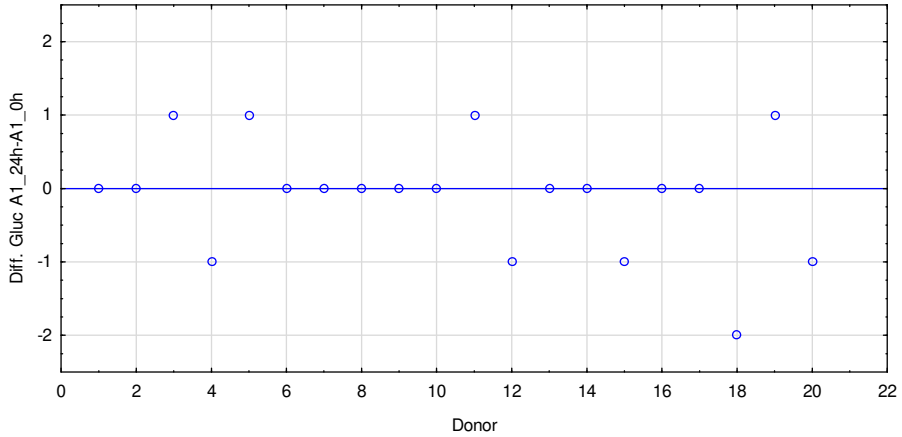


Difference Plot: Gluc [mg/dl] A1_0h/A1_24h



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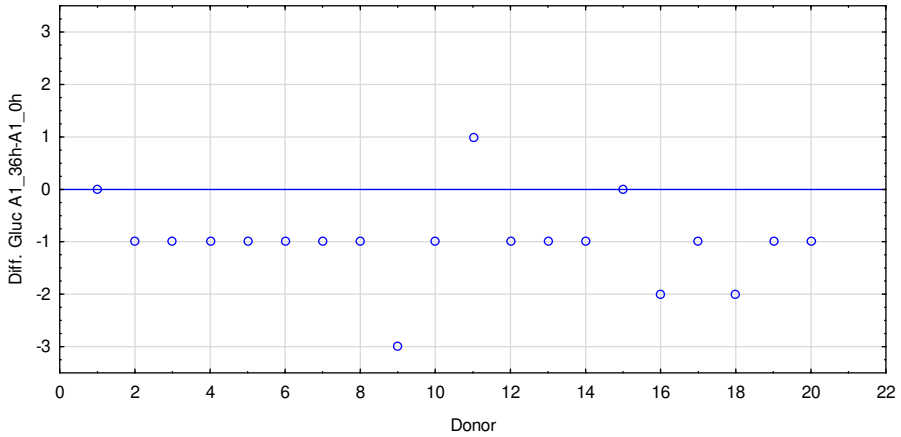

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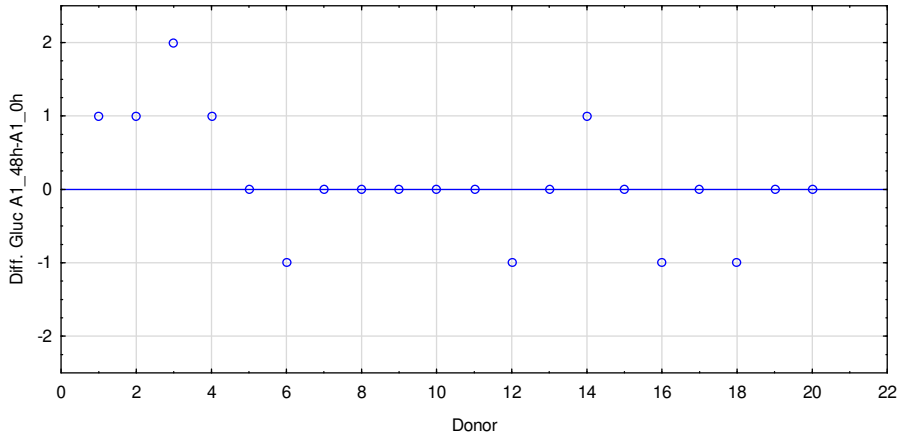

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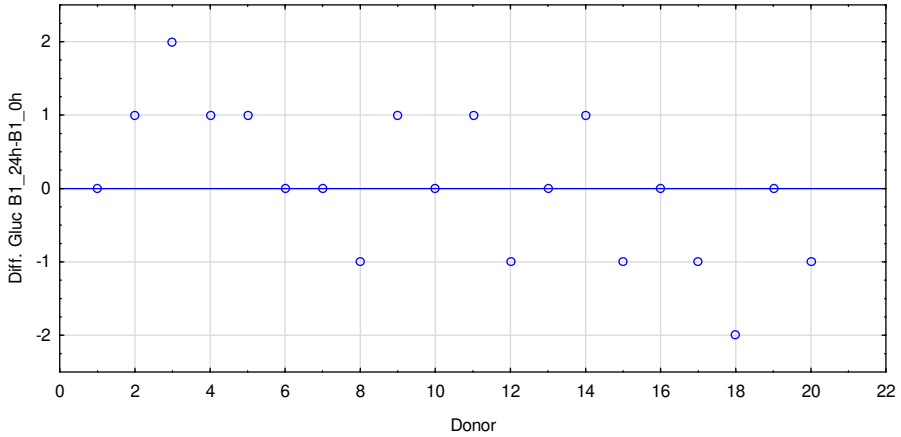

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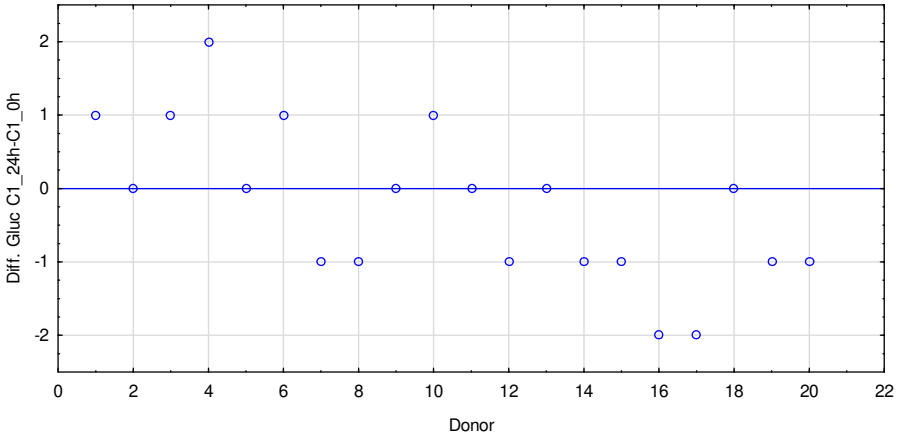

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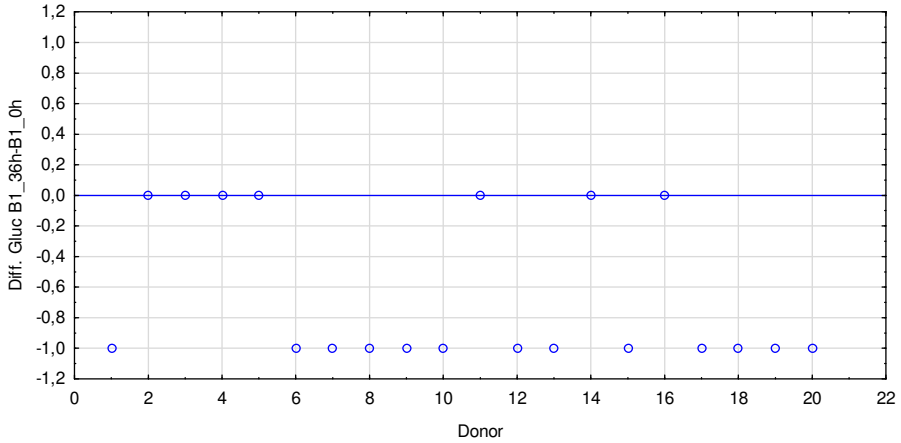

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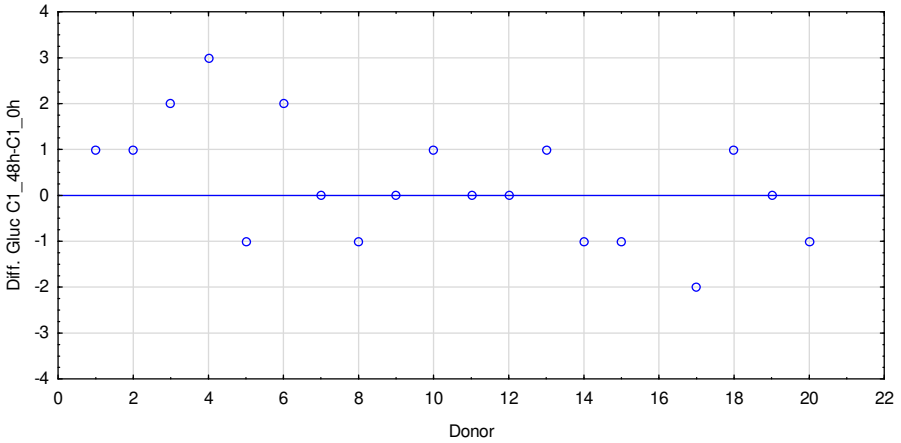

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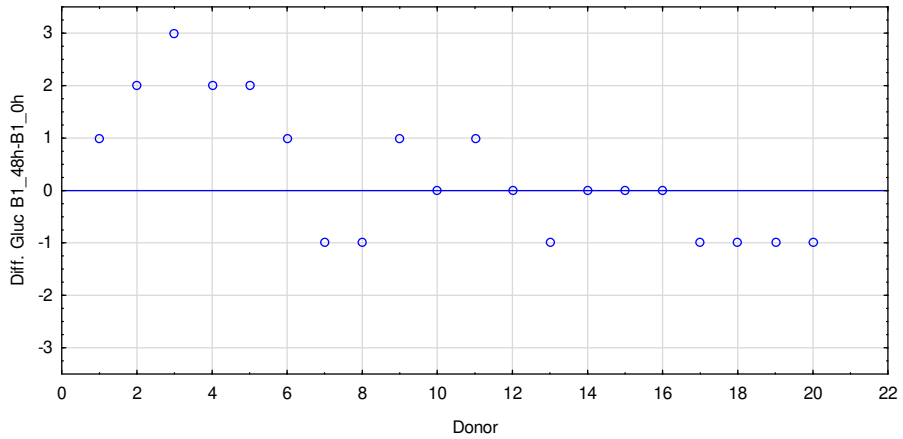

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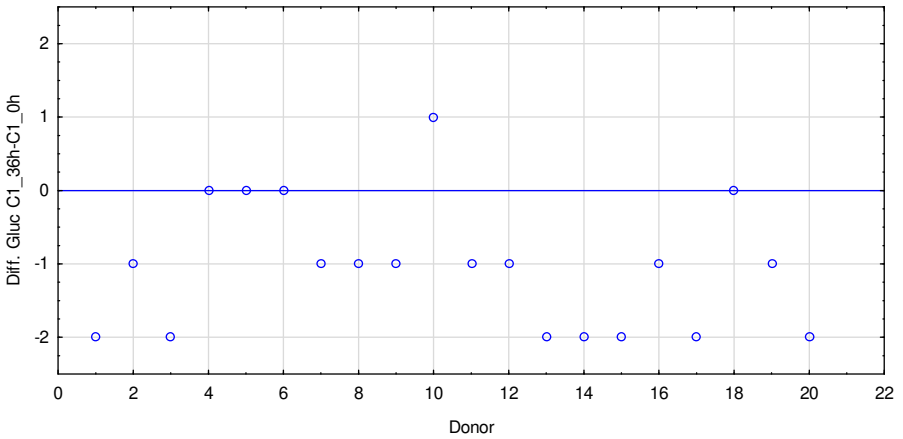
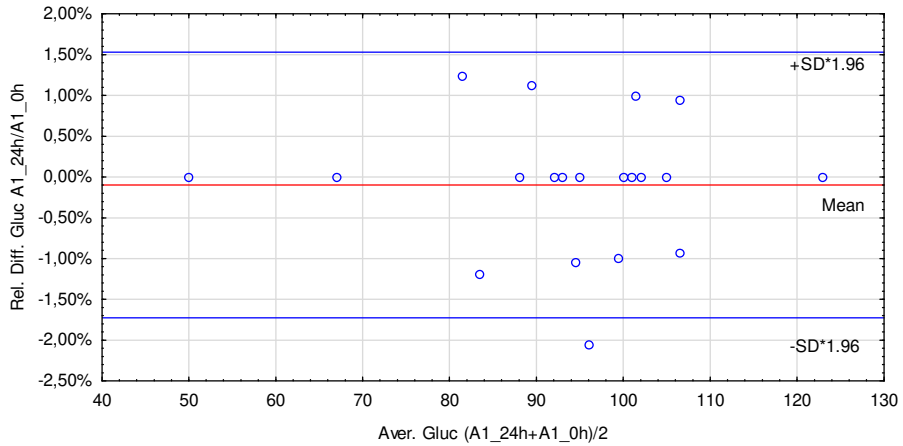


Bland Altman: Gluc [mg/dl] A1_0h/A1_24h



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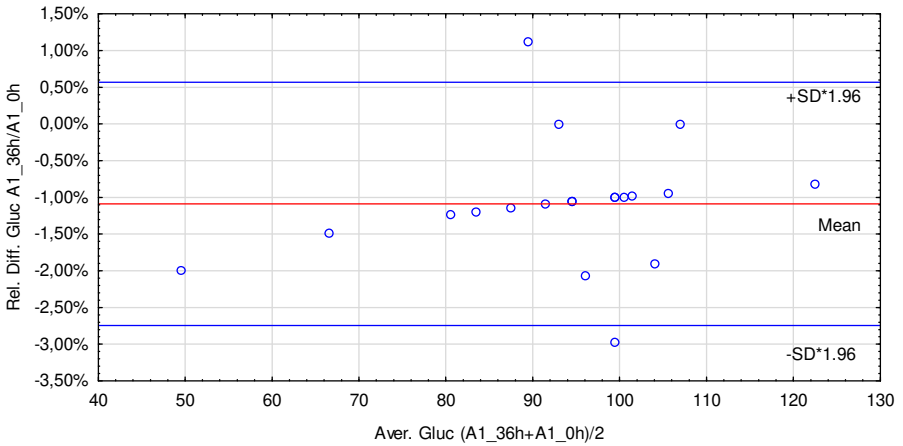

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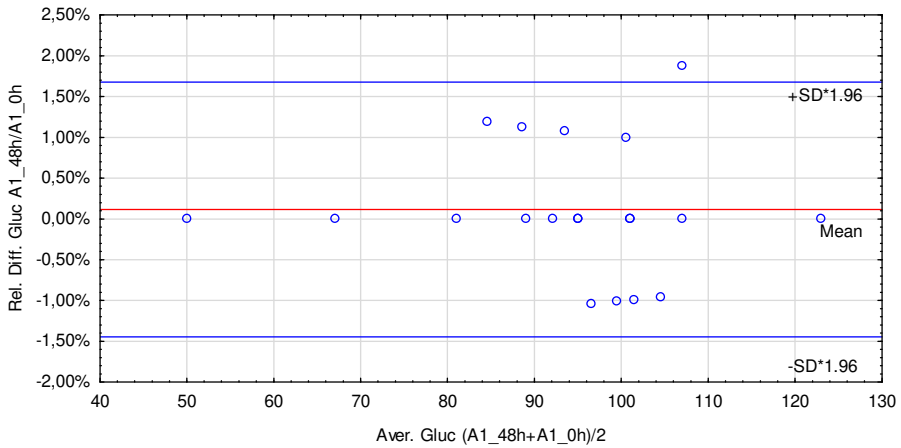

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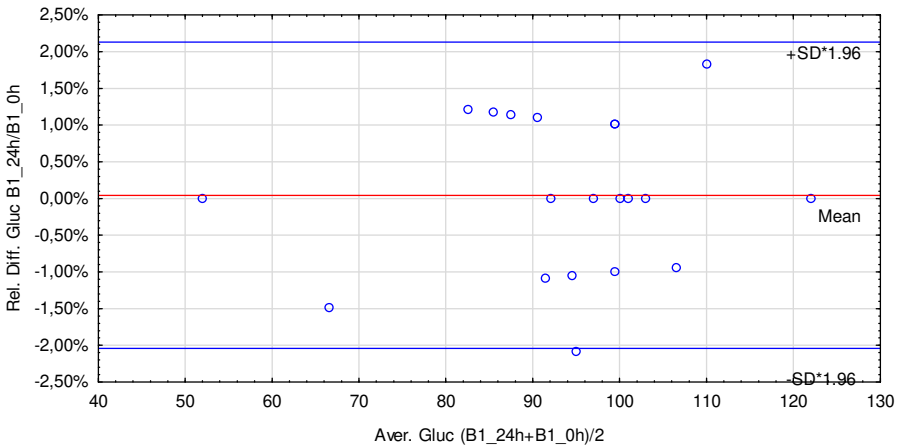

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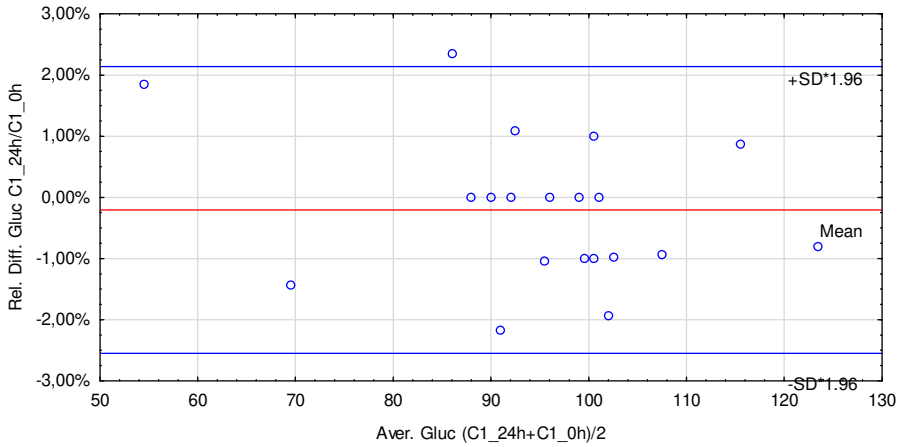

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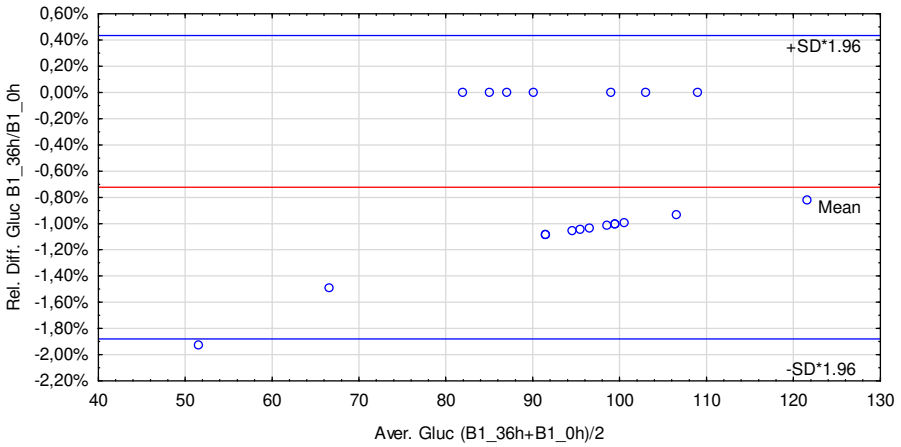

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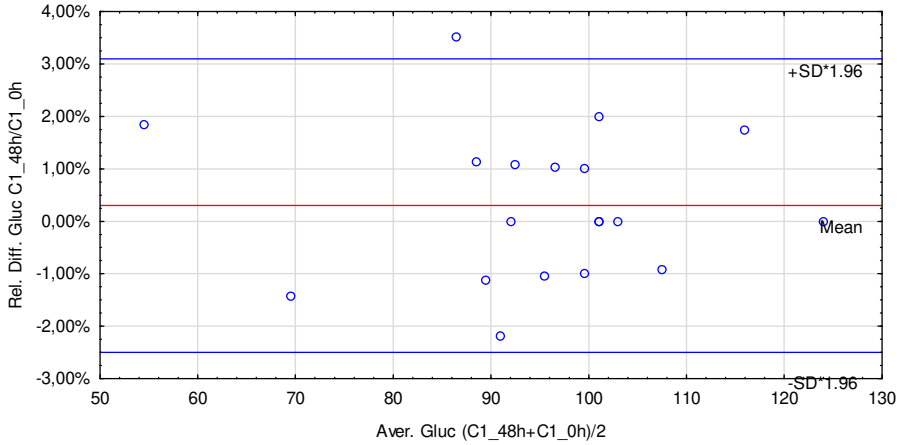

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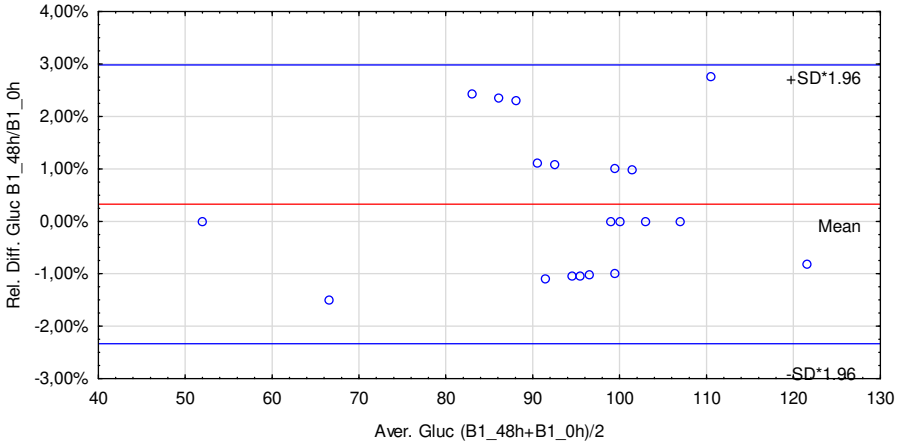

Bland Altman: Gluc [mg/dl] C1_0h/C1_36h