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# HUMAN CARDIAC TROPONIN COMPLEX STABILITY IN VARIOUS MATRICES

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## INTRODUCTION

Ternary troponin complex (ITC) consists of three proteins (troponin I, troponin T, and troponin C (TnC)), and it plays a key role in the regulation of muscle contractions. Specific cardiac isoforms of troponins T (cTnT) and I (cTnI) are used as biomarkers of various heart disorders, including acute myocardial infarction (AMI). During the myocardial damage, different forms of cardiac troponins are released into the blood: full-size ternary complex, low-molecular-weight ternary complex with only the C-fragment of cTnT (LMW-ITC), binary cTnI-TnC complex (IC) (Fig. 1), and various proteolytic fragments of cTnT and cTnI [1, 2].

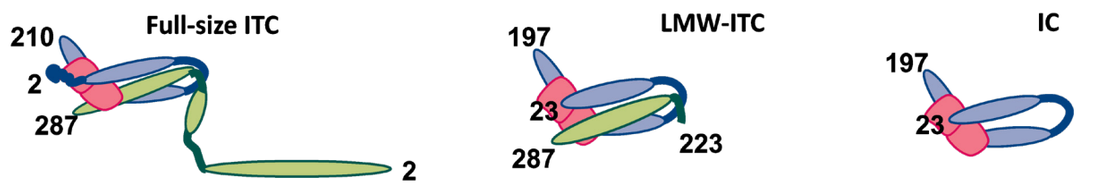


Figure 1. Schematic representation of cardiac troponin complexes that are present in the blood of AMI patients.
*Red: TnC; green: cTnT; blue: cTnI. The numbers designate the terminal amino acid residues [2].*

However, it is not known whether these forms originate from damaged myocardial cells or if the dissociation of the complex also occurs in the patient's bloodstream or in blood samples collected for analysis. In this work, we studied the *in vitro* stability of ITC-complex spiked in various types of blood samples.

## MATERIALS AND METHODS

Native ITC-complex was incubated in different matrices at +4°C, +20°C, and +37°C for up to 24 h and studied by different types of sandwich immunoassays, gel filtration, and Western blotting. Both the anti-troponin monoclonal antibodies (mAbs) and the human native ITC-complex were from Hytest (Finland).

Gel filtration studies (GF) were performed using an AKTA pure chromatography system (GE Healthcare). A total of 1 mL of the ITC-complex dissolved in different matrices was loaded on a HiLoad Superdex 200 PG 16/60 column (GE Healthcare) and 1 mL fractions were collected and analyzed in immunoassays.

Cardiac troponins were measured using various sandwich fluoroimmunoassays (FIA) and chemiluminescence immunoassays (CLIA) specific to different forms of cardiac troponins and troponin complexes (Tcom8-TnT7E7 specific to ITC, TnI84-TnC7B9 specific to IC, TnT155-TnT7E7 specific to free cTnT, and TnC12G3-TnC7B9 specific to TnC). Conjugates of mAbs with stable $Eu^{3+}$-chelate (FIA) and biotin-polyHRP system (CLIA) were used for detection.

Cardiac troponins were detected after immunoprecipitation by means of Western blotting using monoclonal antibodies specific to cTnI (mAb TnI560) and cTnT (mAbs TnT329 and TnT7E7). Conjugates of mAbs with biotin were used for detection.

## RESULTS

### Time course of immunochemical activity of the ITC-complex in different matrices

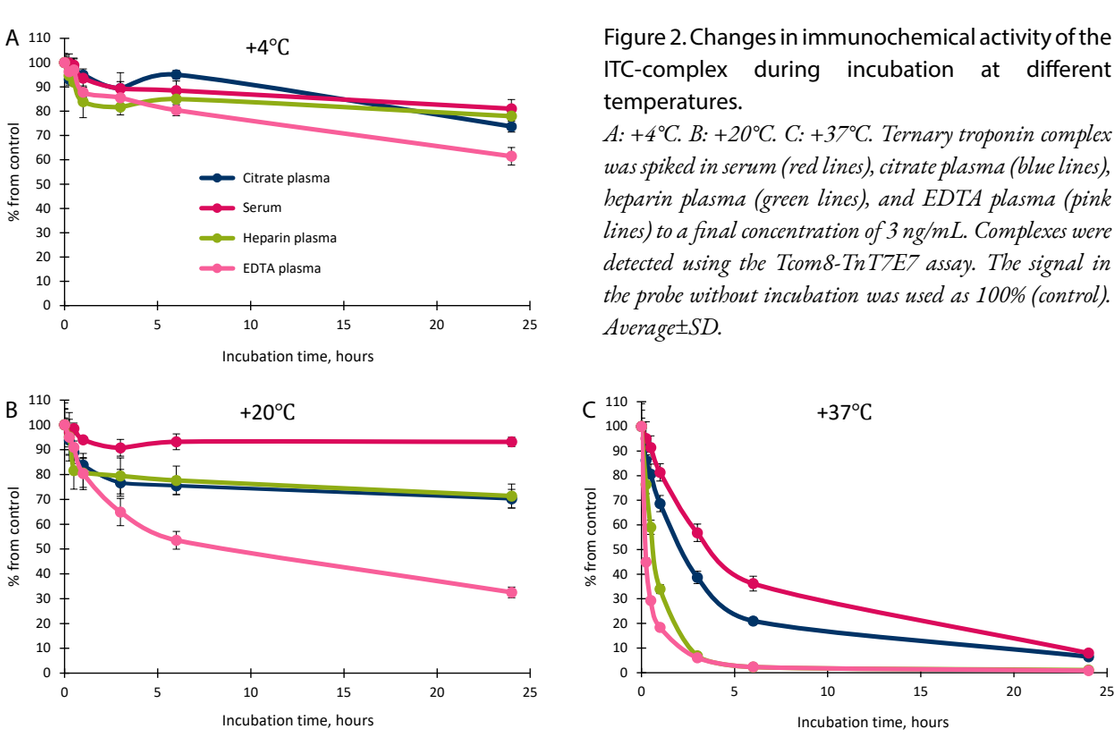


Figure 2. Changes in immunochemical activity of the ITC-complex during incubation at different temperatures.
*A: +4°C. B: +20°C. C: +37°C. Ternary troponin complex was spiked in serum (red lines), citrate plasma (blue lines), heparin plasma (green lines), and EDTA plasma (pink lines) to a final concentration of 3 ng/mL. Complexes were detected using the Tcom8-TnT7E7 assay. The signal in the probe without incubation was used as 100% (control). Average±SD.*

We observed a decrease in the immunochemical activity of the ITC-complex during incubation only at +37°C in serum, citrate, and heparin plasmas with half-lives of 1.0 h, 4.0 h, and 2.5 h respectively.

In EDTA plasma, the ITC-complex is less stable: a half-life of more than 24 h was determined for incubation at +4°C. At higher temperatures (+20°C and +37°C) half-lives are only 6.0 h and 0.3 h respectively.

### Stability of the ITC-complex components




Figure 3. Degradation of cTnI and cTnT after *in vitro* incubation of the ITC-complex in various matrices.
*Ternary troponin complex was dissolved to a final concentration of 3 ng/mL and incubated for 3 h and 24 h. cTnI (A) and cTnT (B) and their proteolytic forms were detected after Western blotting using the TnI560 and TnT329+TnT7E7 mAbs respectively.*

cTnI and cTnT were shown to be stable during the incubation in citrate and heparin plasmas. However, both proteins were partially degraded in serum and EDTA plasmas.

### Dissociation of the ITC-complex in various matrices

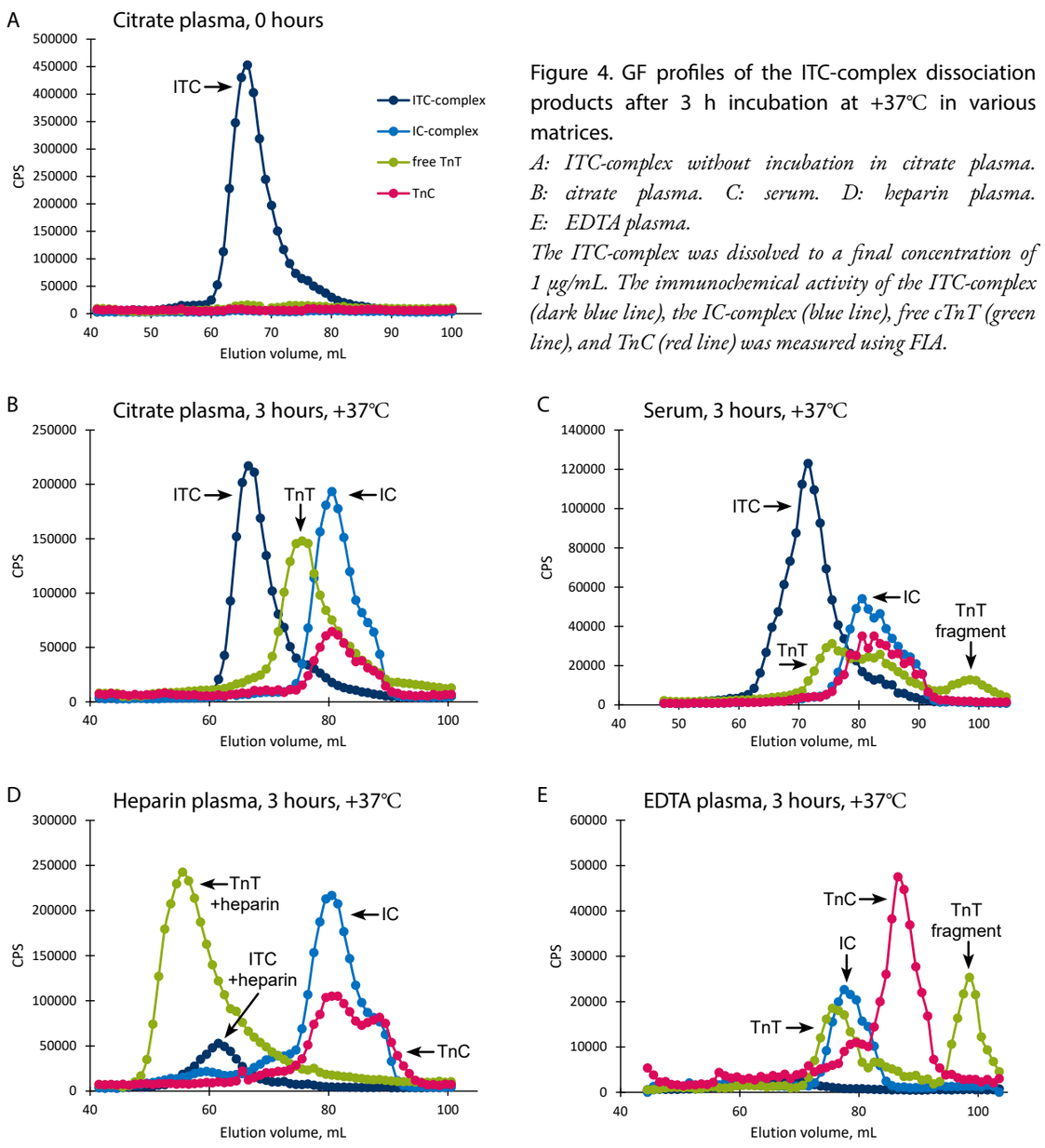


Figure 4. GF profiles of the ITC-complex dissociation products after 3 h incubation at +37°C in various matrices.
*A: ITC-complex without incubation in citrate plasma. B: citrate plasma. C: serum. D: heparin plasma. E: EDTA plasma.*
*The ITC-complex was dissolved to a final concentration of 1 µg/mL. The immunochemical activity of the ITC-complex (dark blue line), the IC-complex (blue line), free cTnT (green line), and TnC (red line) was measured using FIA.*

After incubation for three hours in citrate plasma, a small part of the ITC-complex dissociates to the IC-complex and free cTnT (Fig. 4, B). A similar but much more active process occurs in heparin plasma (Fig. 4, D), resulting in the almost complete dissociation of the ITC-complex within 3 h. The stability of the ITC-complex in serum corresponds to the stability in citrate plasma, while in EDTA plasma the peak of the ITC-complex is no longer detectable after 3 h of incubation at +37°C. In both serum and EDTA plasma, the dissociation of the ITC-complex is accompanied by degradation of cTnT.

### Effect of autoantibodies (AAbs) on the ITC-complex dissociation

It is acknowledged that autoantibodies specific to the ternary troponin complex can be presented in the blood of some human. We studied whether they have an effect on the dissociation of the ITC-complex.

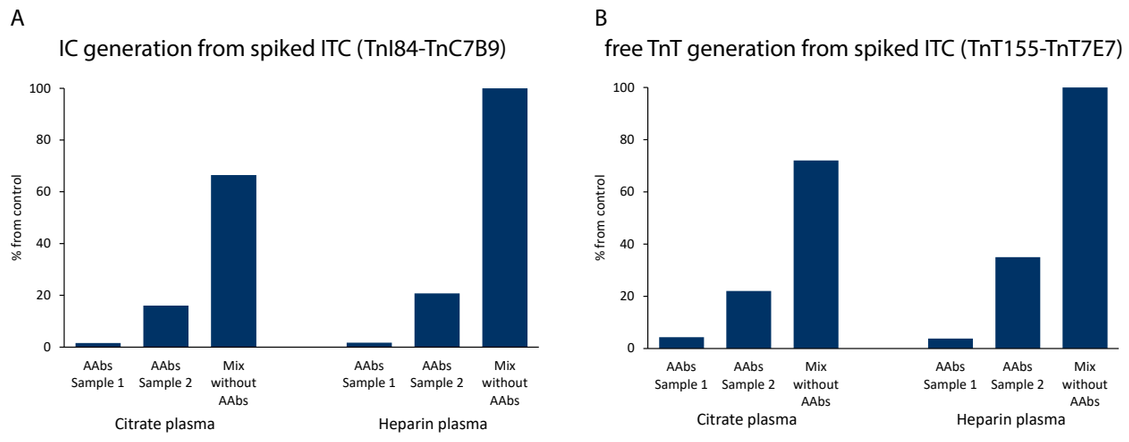


Figure 5. Dissociation of the ITC-complex in the presence of autoantibodies.
*The ITC-complex was dissolved to a final concentration of 80 ng/mL in human plasma samples containing AAbs and plasma mix without AAbs. The immunochemical activity of the IC-complex (A) and free cTnT (B) was measured by FIA, while probes with the ITC-complex spiked in a heparin plasma mix without AAbs were used as controls.*

The formation of free cTnT and the IC-complex is much less pronounced in the presence of autoantibodies. Therefore, it is possible that binding of autoantibodies stabilizes the ternary troponin complex and prevents its dissociation.

## CONCLUSIONS

1. ITC-complex dissociates into binary IC-complex and free cTnT in serum and plasmas during the incubation at +37°C. IC-complex further dissociates in heparin and EDTA plasmas at +37°C to form free TnC.
2. cTnT and cTnI undergo proteolytic degradation in serum and EDTA plasma at +37°C.
3. Anti-troponin autoantibodies prevent the dissociation of cTnT from ITC-complex.

## REFERENCES

1. Vylegzhanina AV *et al.* Full-size and partially truncated cardiac troponin complexes in the blood of patients with acute myocardial infarction. Clin Chem. 2019;65:882–92.
2. Katrukha IA, Katrukha AG. Myocardial injury and the release of troponins I and T in the blood of patients. Clin Chem. 2021;67:124-13.