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FINAL CONFERENCE

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A simple method to analyse bilirubin in animal serum

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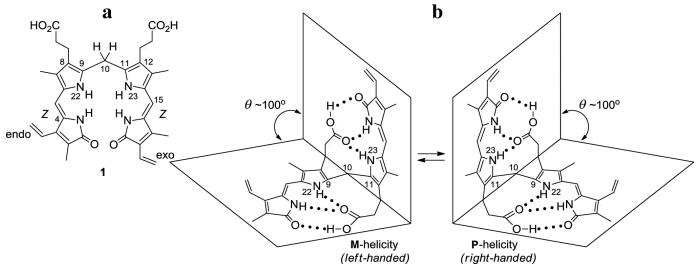
WHAT IS BILIRUBIN? WHY DOES IT MATTER?

- Bilirubin is a molecule synthetised in the cells of our body at the rate of about 300 mg/die.
- Small increases of blood bilirubin have been shown to reduce the risk of several human age-related diseases, including Alzheimer diseases[1].
- **Dietary anthocyanins may cause a small, short increase of serum bilirubin levels[2] , thus contributing to improved health status.**

[1]Ahmed AIA, Driessen S, Van Schendel FME. Role of plasma bilirubin as a biomarker for Alzheimer's disease: A retrospective cohort study. Vol. 62, Journal of the American Geriatrics Society. 2014. 62: 398–9.
[2]Ziberna L, Tramer F, Moze S, Vrhovsek U, Mattivi F, Passamonti S. Transport and bioactivity of cyanidin 3-glucoside into the vascular endothelium. Free Radic Biol Med 2012; 52: 1750–9.



The chemical structure of bilirubin[1]. Intramolecular H bonds (---) determine bilirubin lipophilicity and unusual tri-dimensional conformation.
[1]Ghidinelli S, Longhi G, Abbate S, Boiadjiev SE, Lightner DA. Bilirubin and its congeners: conformational analysis and chirality from metadynamics and related computational methods. Monatshefte für Chemie-Chemical Mon 2019; 150: 801–12.

STANDARD BILIRUBIN ANALYSIS BY THE VAN DEN BERGH REACTION[1].

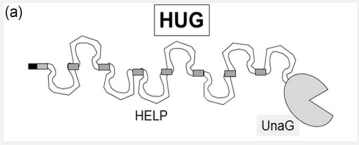
- Fraction A (reaction in water; «direct»): conjugated bilirubin, <10%
- Fraction B (reaction in water + methanol; «total»): conjugated + unconjugated bilirubin
- Fraction C (calculated, Fraction B-Fraction A; «indirect»): unconjugated bilirubin, <90%

[1]Rifai N, Horvath AR, Wittwer C. Tietz textbook of clinical chemistry and molecular diagnostics. St. Louis, Missouri: Elsevier, 2018.

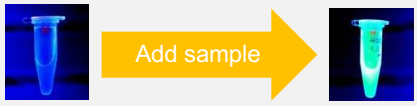
LIMITATIONS & NEEDS

- **The largest fraction of bilirubin is calculated («indirect»)**
  - **Direct analysis of indirect bilirubin is needed**
- **>0.3 mL serum sample is required**
  - **Small sample volume is needed for animal tests**

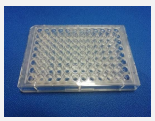
Our methodology



1. HUG is a synthetic protein with a bilirubin-binding unit (UnaG)

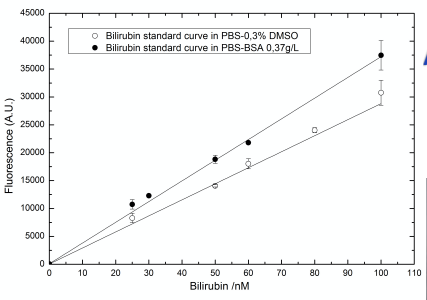


2. HUG binds bilirubin in a sample (< 30 µL) and emits fluorescence



3. Samples are placed in multiwell plates

4. Fluorescence is measured by reader



FIELDS OF APPLICATION

Analysis of serum bilirubin in small sample volumes

- **Small animals (no bleeding)**
- **Humans (one drop of blood)**

Female rats		
serum UCB (µmol/L)	whole blood (µmol/L)	Ref.
average: 0.72 ± 0.05 range: 0.65-0.79	--	*Muraca M. et al., Relationships Between Serum Bilirubins and Production and Conjugation of Bilirubin, Gastroenterology (1987) 92:309-17*
0.67 ± 0.02	0.71 ± 0.05	*our data*
0.81 ± 0.02	0.83 ± 0.04	*our data*

Normal serum contains approximately 96 % bilirubin and 4% of conjugated bilirubin

European population (>18 years) 4.7–24.0 µmol/L total bilirubin
Vitek L., Bilirubin as a predictor of diseases of civilization. Is it time to establish decision limits for serum bilirubin concentrations?, Archives of Biochemistry and Biophysics, 672 (2019) 108062.

our data
Human whole blood : 6.25 ± 0.77 µmol/L

Advantages & Perspectives

- This is a simple method that enables the direct analysis of indirect bilirubin in tiny volumes of blood using universal lab equipment
- **THIS METHOD ENABLES PRE-CLINICAL AND CLINICAL INVESTIGATIONS ON THE IMPACT OF WINE CONSUMPTION ON SERUM BILIRUBIN**

CREDITS, ACKNOWLEDGEMENTS & CONTACTS
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