# UV-C IRRADIATION: A METHOD FOR VIRUS INACTIVATION OF PHYSIOLOGICAL ACTIVE ANIMAL DERIVED BLOOD PRODUCTS!

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**Transfusion medicine**

## What is Acto$_2$Hem®?

Acto$_2$Hem® is a physiological active bovine hemoglobin solution with high ability to bind and release oxygen and is currently in the development as a potent oxygen carrier for biopharmaceutical use.

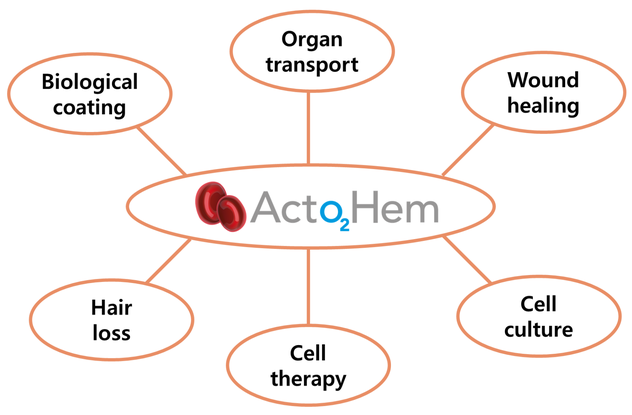


**Figure 1: Possible applications of Acto$_2$Hem®.**

## The importance of virus inactivation

Biological raw material derived from cattle are routinely used for the manufacturing of biopharmaceutical products but are still a major risk for viral contaminations. Therefore, the implementation of a viral clearance and inactivation step in the manufacturing process is essential to prevent viral contamination and to assure product safety[1,2,].

**Table 1: Common viral pathogens present in bovine derived blood products.**

| Virus[a] | Family | Genome | Enveloped | Size (nm) |
|---|---|---|---|---|
| BVDV | Flaviviridae | ssRNA | Yes | 45-55 |
| PI-3 | Paramyxoviridae | ssRNA | Yes | 100-200 |
| Reo-3 | Reoviridae | dsRNA | No | 70-80 |
| BHV-1 | Herpesviridae | dsDNA | Yes | 120-200 |
| BPV | Parvoviridae | ssDNA | No | 18-25 |

**a) Abbreviations used: BVDV = Bovine viral diarrhea virus; PI-3 = Parainfluenza virus 3; Reo-3 = Reovirus 3; BHV-1 = Bovine herpes virus 1; BPV = Bovine parvo virus.**

Previous experiments on Acto$_2$Hem® revealed that gamma irradiation (dose: 25 kGy) and heat treatment (56°C for 2 hours) resulted in a significant increase of methemoglobin (MetHb) up to 65% (data available upon request). Excess levels of MetHb indicate a loss of function of Acto$_2$Hem®, due to the inability of MetHb to transport oxygen. Interestingly, advances in LED technologies brought ultraviolet-C (UV-C) irradiation into focus as an alternative to traditional viral inactivation methods, especially for Acto$_2$Hem®.

## Objectives:

**The aim of this study is to investigate the applicability of UV-C irradiation to a physiological active hemoglobin solution:**

- **The recommendation of UV-C wavelength and sample layer thickness.**
- **Does UV-C irradiation increase MetHb concentration?**
- **Does Hb concentration stay constant during UV-C treatment?**
- **Does Act-O2-Hem lose it's oxygen binding ability?**

## Methodology and Experimental Design:

### UV-C Irradiation

- The germicidal UV-C light is often used in a variety of purification steps for water, air and food disinfection.
- Viruses/Pathogens are inactivated as a result of irreversible formation of pyrimidine dimers due to a photochemical damage to their nucleic acids.
- Unwanted protein damage is minimized by using the wavelength of 255 nm which predominately affects nucleic acids.
- Viruses resistant against heat or acid treatment as well as non-enveloped viruses are sensitive to UV-C Irradiation.
- UV-C irradiation was successfully shown to inactivate viruses in fetal bovine serum[3].

All experiments were performed using the PearlLab Beam from Aquisense Technologies. The system consists of an irradiation unit with three different wavelengths 255 nm, 265 nm and 280 nm. The following experiments were performed at each wavelength over a duration of 30 minutes (see Figure 2).

### Experimental Set up and Design

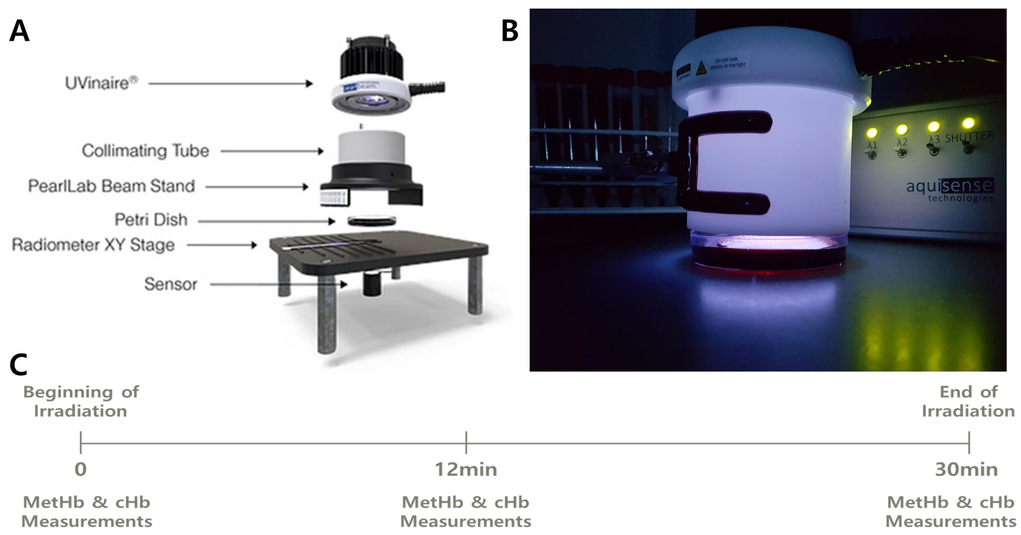


**Figure 2: Overview of the PearlLab Beam system. A)** Illustration of the PearlLab Beam System[4] **B)** Example picture showing UV-C irradiation of Act-O$_2$-Hem sample **C)** Experimental Design of Act-O$_2$-Hem irradiation and measurements for absorption spectra. To fully inactivate pathogens the samples were irradiated for 30 minutes. Control samples were treated equally without irradiation.

## Results:

### 1) UV-C wavelength determination

The quantification of three different wavelength revealed 255 nm as the most suitable one to maintain the stability and purity of Acto$_2$Hem® (Table 2).

**Table 2: MetHb development during UV-C Irradiation at 255, 265 and 280 nm:***

| Wavelength [nm] | MetHB concentration over time [%] | | | |
|---|---|---|---|---|
| | 0s | 300s | 720s | 1800s |
| Control | 2.1 | 2.15 | 2.2 | 2.2 |
| 255 | 2 | 2.7 | 3.35 | 3.9 |
| 265 | 1.55 | 2.25 | 3.15 | 6.4 |
| 280 | 1.45 | 3.4 | 6.65 | 14.35 |

***Data of UV-C irradiation at the wavelength of 265 and 280 nm are available upon request**

### 2) The effect of layer thickness and Hb concentration on UV-C irradiation

The layer thickness and the transmission rate of the UV-C light are essential parameters for successful viral inactivation. Our preliminary results on layer thickness with the PearlLab Beam suggest:

a) Sample layer thickness above 10mm decreases UV-C transmission.

b) Dilution of Acto$_2$Hem® increases UV-C transmission due to a lower sample density but increases MetHb concentration over time.

### 3) The effect of UV-C irradiation with 255 nm on Acto$_2$Hem®

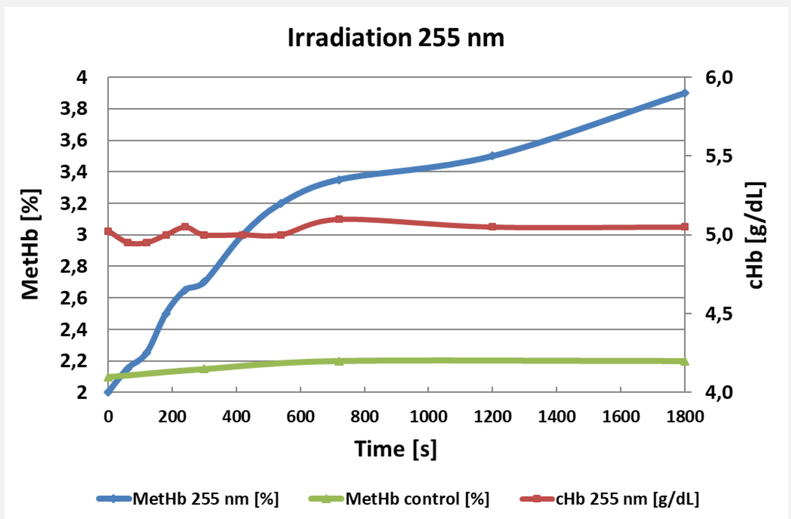


**Figure 3: Concentration of Methemoglobin (MetHb) and Hemoglobin (cHb) during UV-C irradiation:** During UV-C irradiation MetHb content stayed below the quality criteria of 5 % and did not alter cHb concentration.

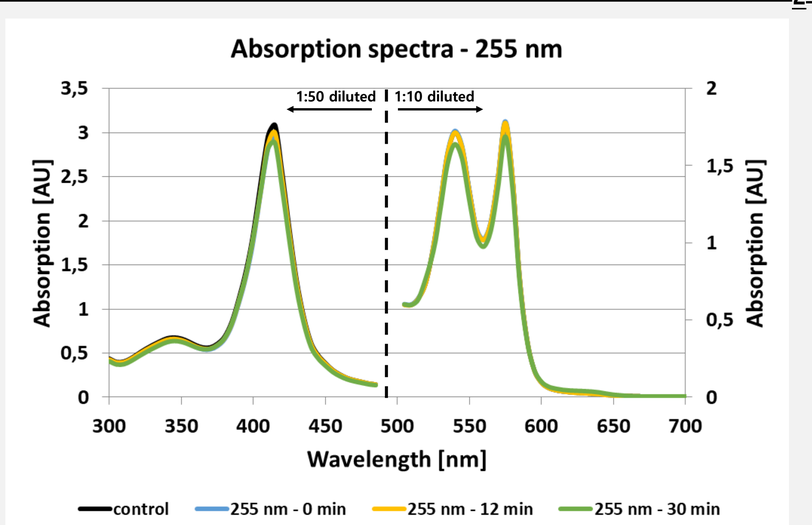


**Figure 4: Absorptions spectra of the samples during the experiment:** Absorbance in the area of oxyhemoglobin showed a slight decrease, whereas absorbance in the range > 600 nm has a minimal increase during irradiation.

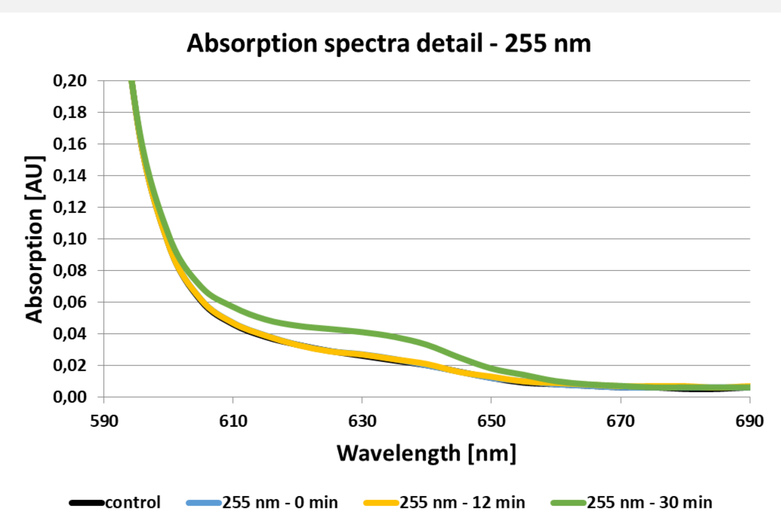


**Figure 5: Detailed absorptions spectra between 550 and 690 nm show the MetHb development during the experiment:** 12 min of UV-C irradiation did not increase absorption of the sample. Only at the end of the experiment (t=30 min) a slight increase in absorption was detected.

## Conclusion:

The aim of this study is to test whether UV-C irradiation is a suitable viral inactivation method for physiological active solutions, like Acto$_2$Hem®. Our first results suggest the following:

- UV-C irradiation with 255nm does not significantly affect functionality, quality and purity of Acto$_2$Hem®.
- The layer thickness of the irradiated solution should be < 10 mm and a dilution of Acto$_2$Hem® increases transmission.
- Our data further strengthens the potential of viral inactivation of animal-derived products by UV-C irradiation as an alternative to traditional methods.
- Viral spiking experiments are essential to fully verify UV-C treatment

All in all, UV-C treatment seems to be a promising method for viral inactivation of Acto$_2$Hem®, therefore further experiments have been planned.

## Future perspectives:

Future experiments, to fully establish and verify UV-C irradiation as a successful method for virus inactivation of a physiological active Acto$_2$Hem® solution, should address the following:

- UV-C irradiation via continuous flow irradiation with a layer thickness between 1 and 5 mm
- UV-C irradiation from both horizontal sides to increase energy transmission rate
- Test multiple UV-C energy doses up to 1000mj/cm$^2$
- Verify successful log reduction and successful inactivation of different viruses most common in bovine herds (e.g. BVDV, PI3)
- Correct implementation of UV-C irradiation in the manufacturing process (e.g. continuous flow UV-C)

## References:

1. Garnick RL. Raw materials as a source of contamination in large-scale cell culture. Dev Biol Stand. 1998;93:21–29.
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3. Vaidya V, Dhere R, Agnihotri S, Muley R, Patil S, Pawar A. Ultraviolet-C irradiation for inactivation of viruses in foetal bovine serum. Vaccine. 2018;36:4215–4221. 2
4. https://www.aquisense.com/pearllab-beam (18.05.2019)

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