



# COMPARATIVE STUDY OF THE USE OF THE TWO-SYRINGE METHOD AND THE PRECELLYS EMULSION KIT

## ANALYSIS OF COLLAGEN-INDUCED ARTHRITIS IN TWO DIFFERENT MOUSE STRAINS

Sorbonne Paris Nord University Li2P INSERM U1125

### CONTEXT

High quality emulsions are key for the success of the experiments in the research field of autoimmune diseases, among many others. However, the most commonly used method for creating emulsions today (two-syringe method), has two important limitations: 1) **reproducibility**, with high variability in the quality of the emulsions obtained between users, and 2) **cost-efficiency**, with a time-consuming procedure that also wastes a large quantity of costly reagents. The **Precellys emulsion kit** has thus been developed to overcome these limitations, and ensure the production of high-quality emulsions consistently.

The **collagen-induced arthritis (CIA)** mouse model is the most commonly studied autoimmune model of **rheumatoid arthritis (RA)**. CIA can be induced in susceptible mouse strains by immunization with an emulsion of Freund's adjuvant and type II collagen (CII). The onset of arthritis usually takes 3 to 5 weeks, and the incidence of the disease typically varies from 50% to 100%, depending on the mouse strain and the CII type.

This Application Note, presents the results obtained by researchers of Sorbonne Paris Nord University (INSERM U1125 Laboratoire Physiopathologie, cibles et thérapies de la polyarthrite rhumatoïde), in which a comparison between the quality of the emulsions created by the conventional two-syringe method and the Precellys emulsion kit is made. The evaluation is based on the onset, incidence and clinical scores of CIA for two different mouse strains.

### MATERIALS

- Precellys Minilyls (Bertin Technologies, Montigny-le-Bretonneux, France)
- Precellys Emulsion kit (Bertin Technologies, Montigny-le-Bretonneux, France)
- Freund's adjuvant
- Type II collagen (CII)
- Mice (C57Bl/6 and DBA/1 strains)





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## PROTOCOL

To compare the ease of preparation of an emulsion and the effectiveness of immunization in the CIA model, identical emulsions were prepared using the classic two-syringe method manually, and the Precellys emulsion kit method with the Minilys. Furthermore, two different mouse strains were immunized in order to compare the efficiency of the immunization: the C57Bl/6 and the DBA/1.

For the C57Bl/6 mice, the comparison was made between two groups of mice with two different types of emulsions (8 mice per group – 32 mice in total). For the DBA/1 mice, the comparison was carried out between two groups of mice with only one type of emulsion (4 mice per group – 8 mice in total).

- Day 0: Preparation of the emulsions with the two methods. All groups of mice were immunized.
- From Day 22: Arthritis readings 3 times a week.
- Day 23: A booster dose of immunization was carried out.
- Day 40: Stop of arthritis readings.

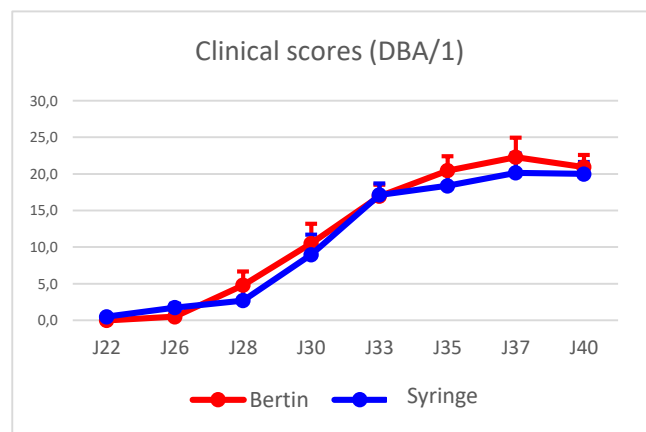
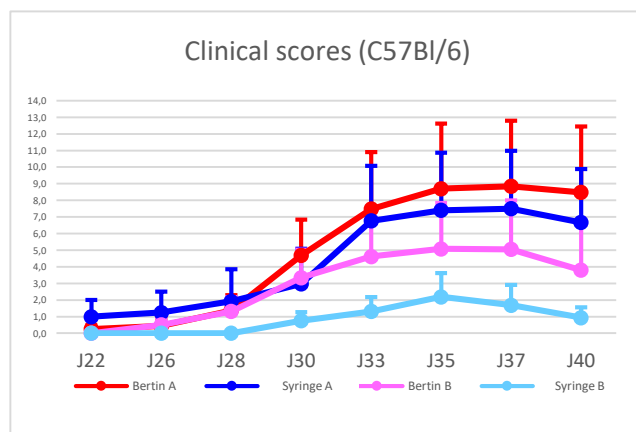
The same protocol was followed for the two different strains of mice (C57Bl/6 and DBA/1).

## RESULTS

The most commonly used method for assessing the severity of CIA is a semi-quantitative clinical scoring system, based on the degree of inflammatory responses in the paws and joints.

For the groups of C57Bl/6 mice, the emulsions prepared with the Precellys emulsion kit gave a lower onset (up to 2 days difference), higher incidence of arthritis and a higher clinical score than with the conventional two-syringe method. The Maximal clinical arthritis index (Amax) obtained with the emulsions created with the Precellys emulsion kit was also higher for the two different emulsions.

For the groups of DBA/1 mice, on the other hand, the levels of incidence of arthritis and the clinical scores were similar between both methods. Nonetheless, the Amax observed was also higher in the case of the emulsion created with the Precellys emulsion kit and the Minilys.



Differences in the clinical scores of collagen-induced arthritis obtained after immunization of two mouse strains (C57Bl/6 and DBA/1 – left and right figures, respectively) with the conventional two-syringe method and the Precellys emulsion kit with the Minilys.



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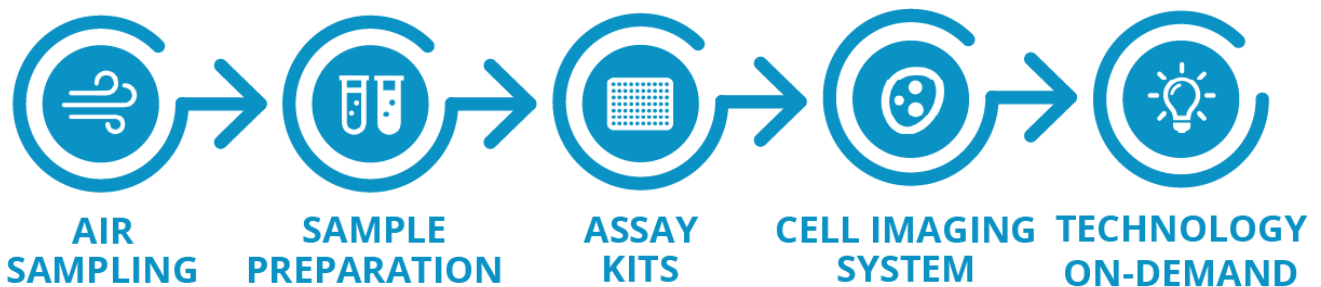
## CONCLUSION

Emulsion preparation for mice immunization to induce arthritis is better with the Precellys Emulsion kit and the Minilys than with the two-syringe method. Particularly in the case of C57Bl/6 mice, for which arthritis appeared earlier and had a higher clinical score.

The emulsion prepared with the Precellys Emulsion kit and the Minilys is of better quality. It seems that the emulsions prepared with the Precellys Emulsion kit are more reproducible over the long term.



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