How to scale up a method from low throughput to high throughput for the quantification of 16 nucleosides? Lesson from experience.

Quentin V andoolaeghe, Valérie Bouchart, Isabelle Vaudorne, Raphaël Delépée

To cite this version:

Quentin V andoolaeghe, Valérie Bouchart, Isabelle Vaudorne, Raphaël Delépée. How to scale up a method from low throughput to high throughput for the quantification of 16 nucleosides? Lesson from experience.. Congrès ANALYTICS 2022, Sep 2022, Nantes, France. hal-03846446

HAL Id: hal-03846446
https://normandie-univ.hal.science/hal-03846446
Submitted on 10 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
How to scale up a method from low throughput to high throughput for the quantification of 16 nucleosides? Lesson from experience.

**Context:** Agriculture, pesticides and cancer

- Link between occupational exposures and cancers demonstrated in farmers
- Molecular epidemiology approach
- Investigation on biobanks samples
- Important statistical power needed

**Pesticides exposure effects?**
- Other determinants?

**Agricultural cohort with exposure description and tasks listing**

**Biobank made with**
- 795 individuals at initial sample time in Calvados department area with blood and urine samples
- 300 follow up after 10 years

**Working in**
- Field Crop
- Bovine breeding

**Exposure description by epidemiological questionnaire**

---

**Plausible mechanism of cancerogenicity**

2 cancerogenicity mechanisms possible studies through analysis of epigenetic and genotoxic alteration

- Oxidative stress
  - 12 Oxidation adducts investigated

- Epigenetic
  - 4 Epigenetic alterations investigated

**Analytical method**

- All solution additions are made with an Integra Assist+ pipetting robot
- For each 96 well plate, 8 calibration points, 2 blanks, and 10 quality check are prepared, according to ICH M10 recommendation

**Sample preparation**

- **Reduction**
  - Heat stability separation with Maizent®-Nagel® extraction kit
  - 50 µg of DNA obtained
  - Reduction
  - Enzymatic hydrolysis

- **Precipitation**
  - Cold ethanol 1 V
  - 50 mL NaCl 0.1mol/L
  - Vortex agitation
  - Centrifugation

- **Washing**
  - 1 Washing
  - 1 mL Ethanol (70:30)
  - Plate agitator
  - Liquid disposal

- **Hydrolysis**
  - 0.2 additions of enzyme solutions
  - 0.2 hours agitation at 37°C
  - 10 µl of HCl

- **Centrifugation**
- **Transfer into LC vial**

- **Evaporation**
  - 1 mL Etoh:H2O (70:30)
  - 1 Washing
  - 1 mL Etoh:H2O (70:30)
  - Plate agitator
  - Liquid disposal
  - Evaporation

- **Precipitation adapted to room temperature to be possible with the pipetting robot**
- Better yield with 1 washing than 2 Evaporation allow to clear the samples of the remaining ethanol

---

**Conclusion and current progression**

- Optimisation for the preparation of 380 samples / week
- Analysis of the 795 agricultural workers from the biobank
- Association of DNA adduct profiles with occupational exposure to pesticides
- Already more than 600 samples extracted
- First analysis currently ongoing

---