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Societal needs

# Validation and implementation of an ELISA for C-peptide analysis in Cameroon

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# Introduction (1) – Diabetes

- Prevalence of diabetes will double in sub-Saharan Africa
- Preventing/managing of diabetes is thus warranted
- Proper classification is required for appropriate treatments

**2010**

**12 million**




**2030**

**24 million**

IDF – Mbanya *et al* Lancet, 2010

# Introduction (2) – Diabetes

## Beta cell mass fails to provide sufficient insulin for glucose homeostasis

## Measuring the beta cell function

- **Important for**
  - **Classification of diabetes**
  - **Following progression of diabetes**

- **Beta cell peptides: proinsulin, insulin & C-peptide**

# Introduction (3) – C-peptide

## Advantages

- Determination possible in insulin treated patients
- Longer plasma half-life (~30 min) than insulin (~4 min)

## Immunoassays

- Currently, C-peptide has to be analyzed abroad
- **Implementation of a cost-effective C-peptide method is needed**
  - Good analytical performance
  - Performed manually
- ELISA is an affordable method

# Aims

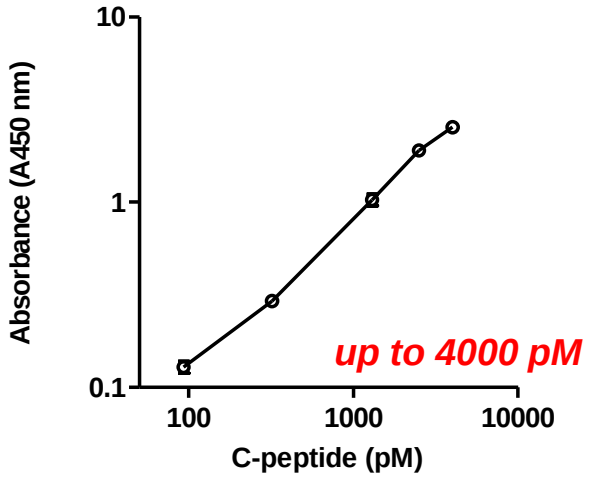
1. To validate the human C-peptide normal sensitive ELISA kit from Mercodia

2. To implement the kit in Cameroon (Biotechnology Centre, University of Yaounde 1)

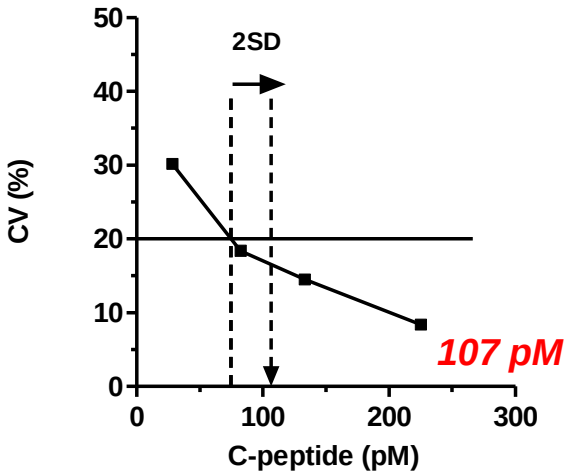
3. Technology transfer: training of technical staff in Yaounde

# Analytical validation (1)

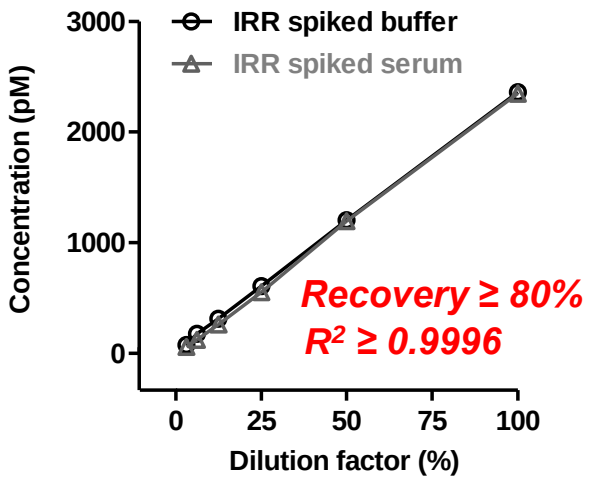
## A Calibration curve



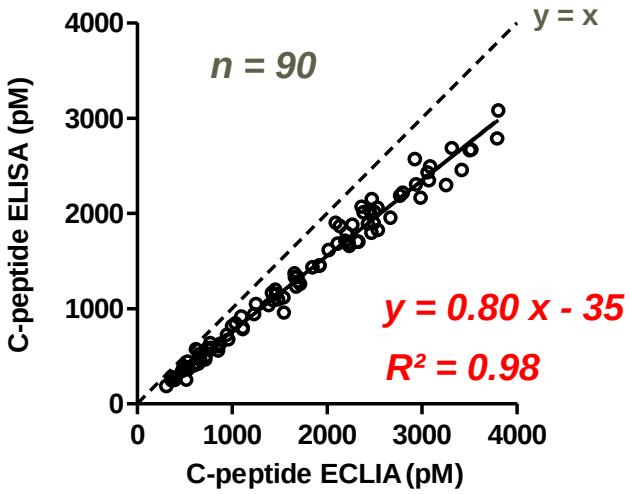

## B Functional sensitivity




## C Dilution linearity




## D Method comparison



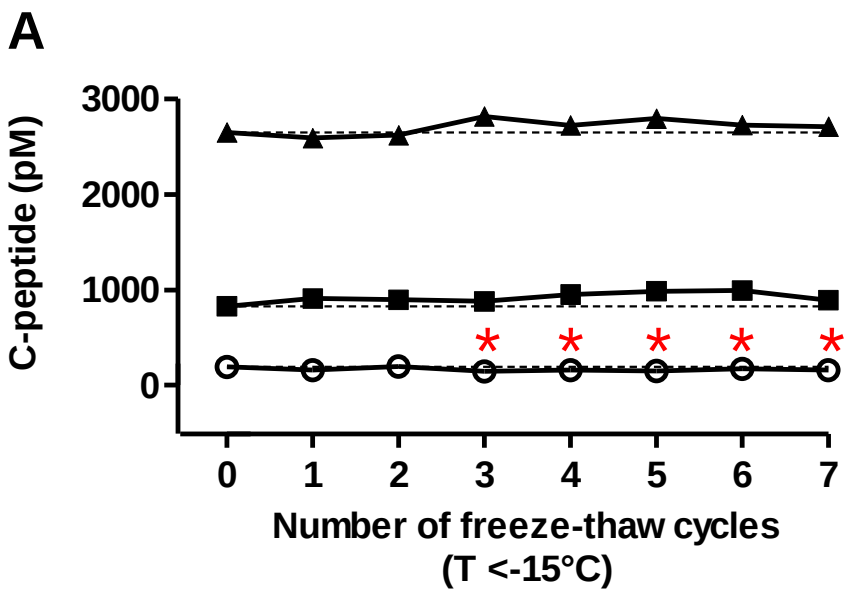

IRR: International Reference Reagent for human C-peptide

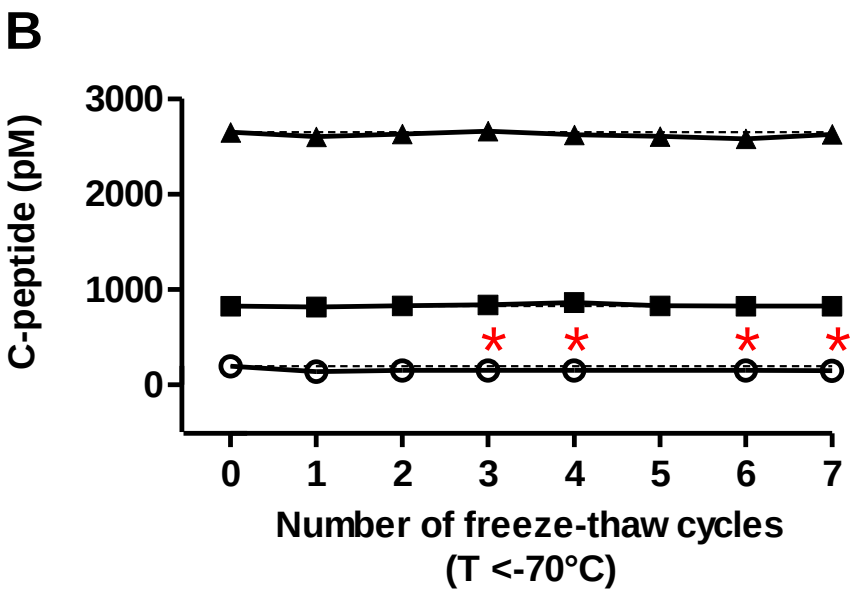
# Analytical validation (2)

| Analytical parameter | Results |
|---|---|
| **Cross-reactivity** | **0% for human proinsulin** |
| **Interference** | **<10%** |
| * Hemoglobin | up to 2 - 5 g/L |
| * Intralipid | up to 20 g/L |
| * Bilirubin | up to 0.3 g/L |
| * Heterophilic antibodies | for 10/12 samples |
| **Precision** | |
| * Intra-assay | 5.2 - 9.4% |
| * Inter-assay | 2.9 - 9.9% |
| * Total error | **4.6 - 12.0%** |
| **Accuracy** | |
| * Serum | **81 - 94%** |
| * Buffer | **93 - 98%** |

# Stability study (1)

## Effect of multiple freeze-thaw cycles




* $|100 \cdot (\dot{z}_{stored} - \mu_T)/ \mu_T| > 20$ with $\mu_T$ = nominal concentration

# Stability study (2)

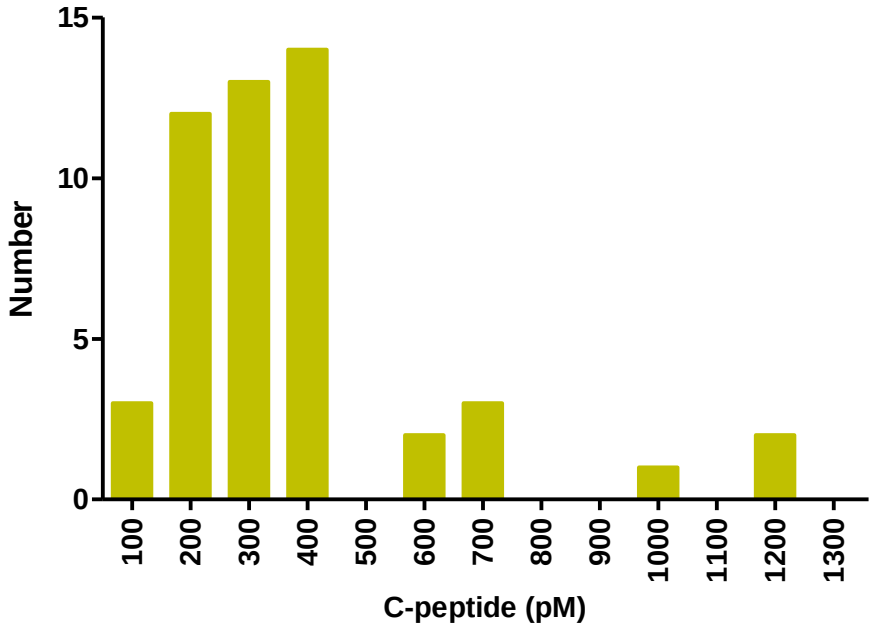
**Stability of analyte in plasma at room temperature (20-25 C) after 24-h of storage**

| | C-peptide | Bias** |
|---|---|---|
| Low | <333 pM | -3 to 11% |
| Medium | 333-2000 pM | -5 to 8% |
| High | >2000 pM | -2 to 11% |

** $|100 \cdot (\dot{z}_{stored} - \mu_T)/ \mu_T| \leq 20$ with $\mu_T$ = nominal concentration

# Reference range for Cameroon

**Analysis of plasma C-peptide of non-diabetic Cameroonian subjects (n=50) with fasting glycemia levels of 4.0 - 5.4 mM.**




| | C-peptide |
|---|---|
| Median | 326 pM |
| P2.5 – P97.5 | 127 – 1209 pM |

# Implementation in Yaounde (1)

**September 2012**

**One week training of technical staff**

**(Biotechnology Centre, University of Yaounde 1)**



**Training group and organizers**



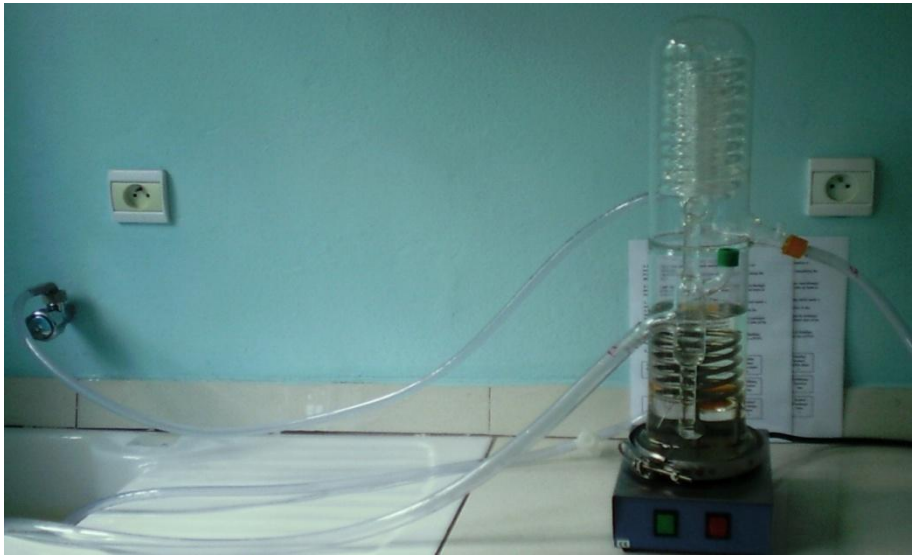
**Bench-top manipulation of the Mercodia kit**

# Implementation in Yaounde (2)

**September 2012**

**One week training of technical staff**

**(Biotechnology Centre, University of Yaounde 1)**



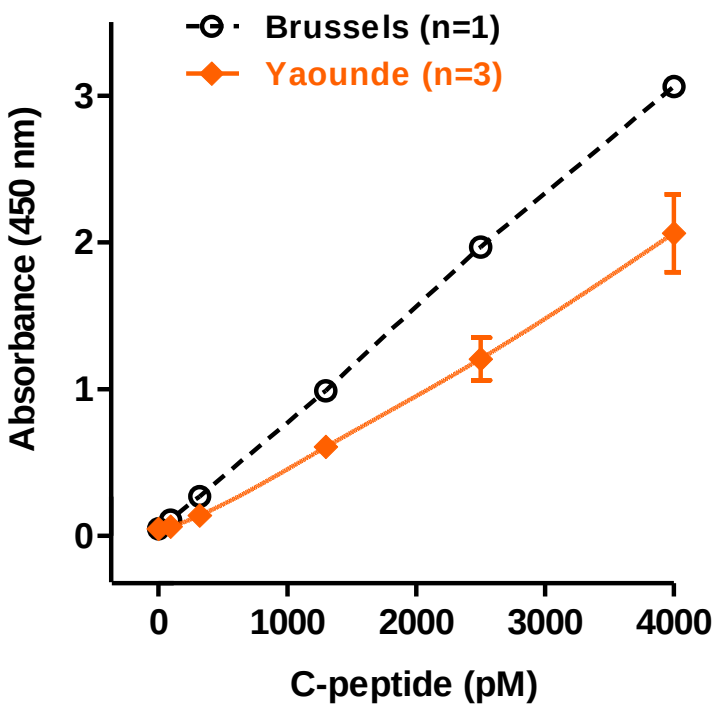
**Distiller**



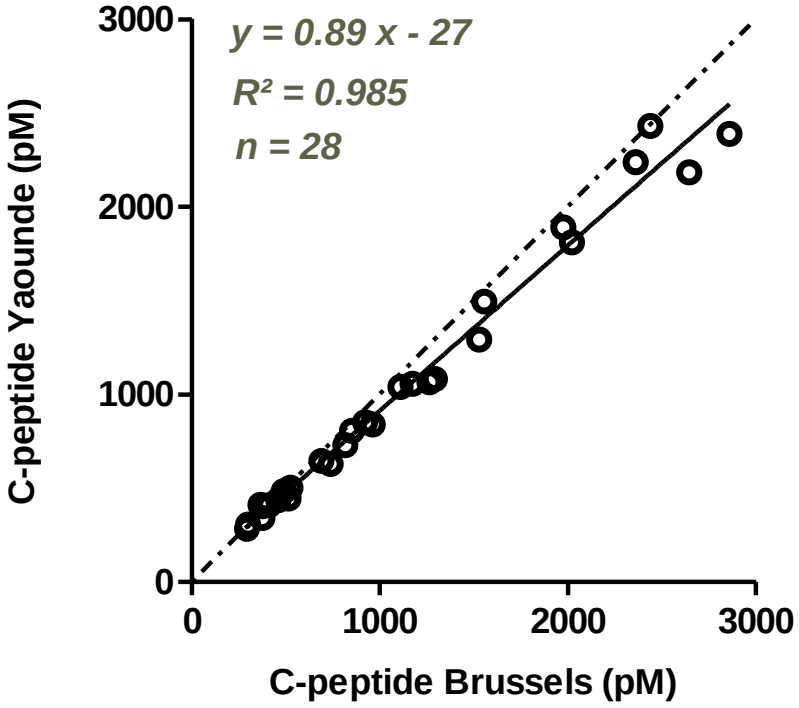
**ELISA plate reader**

# Implementation in Yaounde (3)

## Calibration curve




## Method comparison



# Conclusions

- **The Mercodia C-peptide ELISA kit has been successfully validated**
- **Relatively low investments are needed for this method; it can completely be performed manually; it is suitable for low-income countries**
- **Preliminary data show a good correlation between C-peptide levels analyzed in Yaounde & Brussels**
- **Regular but short electric power failures in Cameroon may not compromise data of C-peptide in plasma samples (C-peptide is stable 24-h at room temperature)**

# Prospects

- **Quality follow-up for a sustained performance over time**
- **To measure beta cell function of diabetic individuals in Yaounde**
- **To implement the C-peptide method for all diabetic patients in Cameroon to optimize treatment**
- **To improve diabetes classification by using biological markers (such as C-peptide) in addition to clinical criteria**

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# Thanks for your attention !