Barking, Havering and Redbridge University Hospitals NHS Trust

Optimisation of Sebia IT/IF control for Serum Proteins Capillary Electrophoresis

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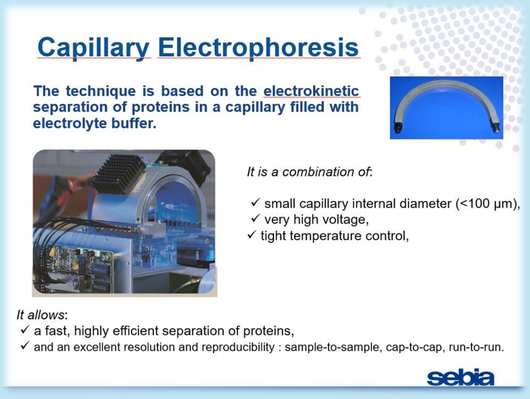
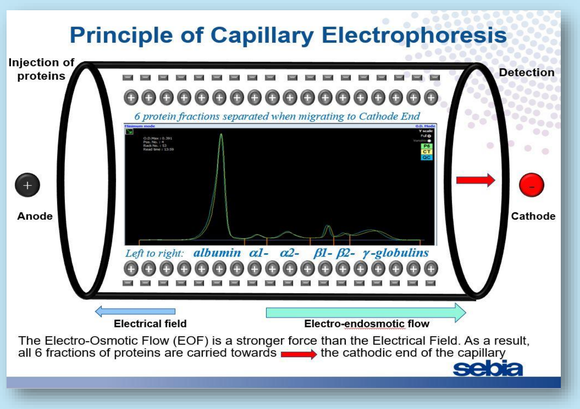
KEY WORDS

Paraproteins, Positive IQC, Qualitative, Quantitative, Serum Proteins Capillary Electrophoresis

INTRODUCTION

The Sebia Capillarys 2 Flex (CZE2) analyser is a well-established equipment used in many U.K. clinical laboratories for the detection of monoclonal **paraproteins** in myeloma patients [1].
Using the **Capillary** Zonal **Electrophoresis** (CZE) principle [2], its Protein E(6) assay program separates **serum proteins** into 6 fractions: albumin, α1 & α2, β1 & β2, and γ-globulins.





The Sebia IT/IF Control (Ref Number 4788) is an internal quality control (**IQC**) designed as a **positive qualitative** marker for the identification of paraproteins but unlike its more widely used sister IQC (the Normal IQC, Ref Number 4785/4786), it has no **quantitative** data on its factsheet.

But quality control guidelines* would demand a good quantitative positive IQC to be found:
*"*Each Lab must design IQC systems which verify the success of the intended quality results.*"
"The Lab has to provide the levels of QC materials run each day, the frequency of performing the QC, the types of QC materials, and the QC acceptance criteria." **source: ISO 15189 : 2007**
""The Lab ought to use more than one level of IQC materials, i.e. normal & pathological/abnormal control. For each IQC material each lab should establish its own mean."*
Source: https://clsi.org/standards/

For this reason, between 2019 and 2024 a study was carried out on our CZE2 analyser to explore the quantitative potential of two lots of Sebia IT/IF Control (lot 13098 & lot 09121, respectively).

METHODS

Preparation of IT/IF IQC:

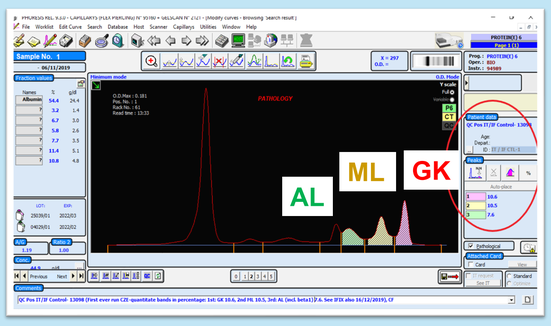
Buy & keep enough of each lot of Sebia IT/IF Control for 1½ - 2 years. Store at 4°C until expiry date. Reconstitute a lyophilized bottle with 1mL deionised water. Aliquot 100µL to vials and freeze at -20°C. Thaw to run on CZE2 analyser once a week.

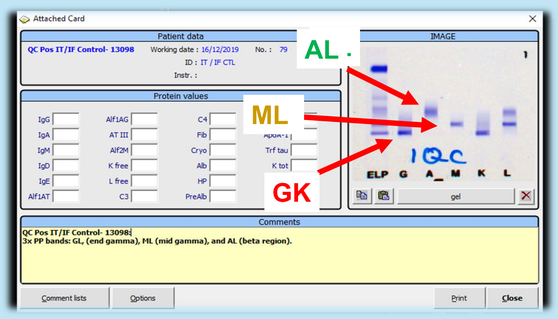
Steps for Verification and Optimisation of IT/IF IQC:

For each lot, use immunotyping techniques (either Capillarys immunosubtraction or Hydrasys immunofixation or both) to verify the identities of the Paraproteins bands.
Measure peak area of each band in %. Convert % area to gram mass *(would need Total Protein (TP) value in gram mass of the IQC*).
Collect minimum of 20 data of each paraprotein band to establish preliminary mean and SD and CV.
Set up statistics on any existing software program. Monitor progress over time.

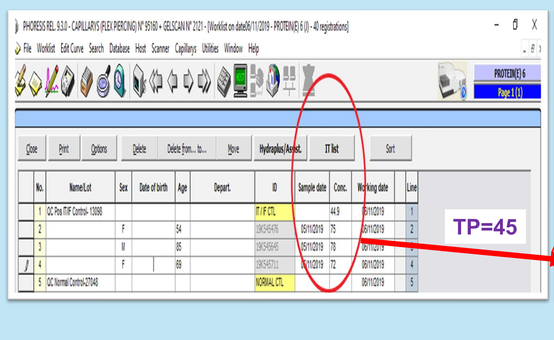
RESULTS

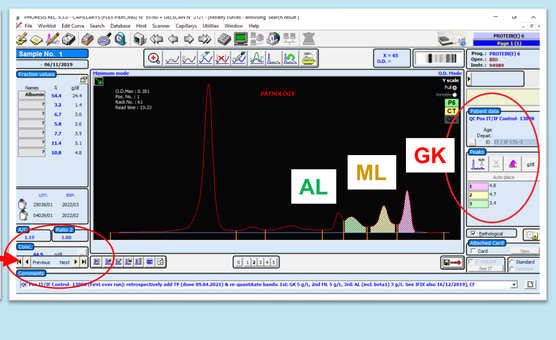
Sebia IT/IF Control Lot 13098 – 3 paraproteins (IgGK, IgML & IgAL) confirmed & quantified in % area on CZE2 *(below left);* verified by Immunofixation on Hydrasys2 *(below right)*





Input TP (g/L) into Phoresis software to convert paraprotein band from % area to gram mass

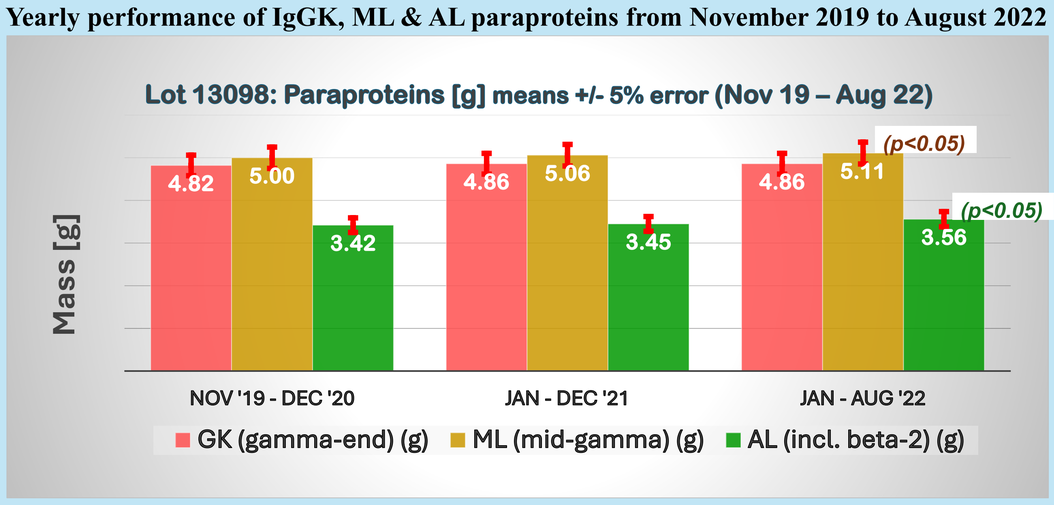




Paraproteins ID, their respective means (in grams), 2xSD, and CV (%) for Sebia IT/IF Lot 13098 (122 data collected between 11/2019 & 08/2022; expired 09/2022):

IgGK (γ-end): mean 4.9g 2SD 0.2g CV 2.3% ; IgML (mid-γ): mean 5.1g 2SD 0.3g CV 3.3% ; IgAL (including β2): mean 3.5g 2SD 0.3g CV 4.0%.

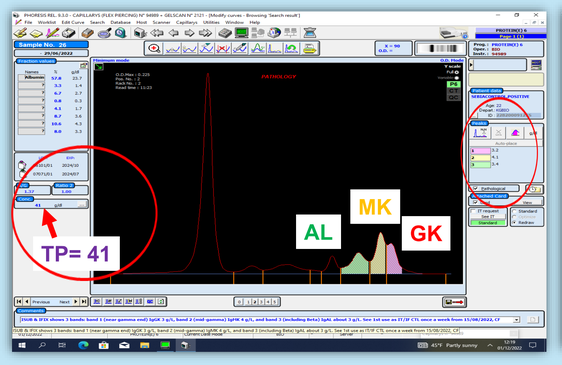
Yearly performance of IgGK, ML & AL paraproteins from November 2019 to August 2022

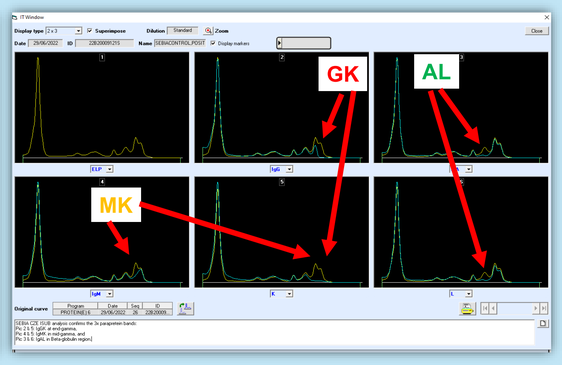


RESULTS *(continued)*

Sebia IT/IF Control Lot 09121 (expiry date 12/2025)

3x paraproteins (IgGK, IgMK & IgAL) confirmed & quantified in (g) *(below left)* and verified by immunosubstraction on CZE2 *(below right)*

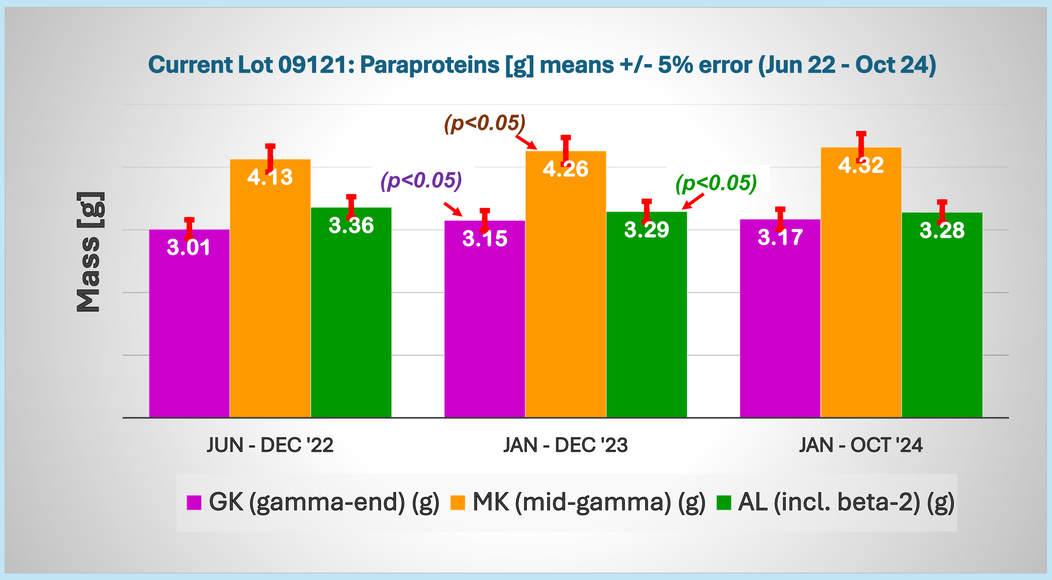




Paraproteins ID, their respective means (in grams), 2xSD, and CV (%) for current lot 09121 (129 data collected between 06/2022 & 10/2024; expiry date 12/2025) are:

IgGK (γ-end): mean 3.1g 2SD 0.3g CV 5.1% ; IgMK (mid-γ): mean 4.3g 2SD 0.4g CV 4.7% ; IgAL (including β2): mean 3.3g 2SD 0.3g CV 4.3%.

Yearly performance of IgGK, MK & AL paraproteins from June 2022 to October 2024 in gram mass



DISCUSSIONS

Two lots of Sebia IT/IF Control were showcased: each have three distinct paraproteins well separated across the β– and γ-globulin region. Each band can be quantitated in gram mass; their means over a three-year period are consistent, all with a CV under 6%, thus demonstrate their good stability, reproducibility & precision.

Some of these paraproteins in both lots shifted (mostly increase) from one year to another. While some of these shifts were statistically significant (where indicated on the Histograms shown above: with *p value < 0.05*, Two-Tailed t-Tests), they are not clinically significant because the minimum reporting threshold in this laboratory is down to 1 gram.

CONCLUSIONS

Qualitatively:
Not one but three paraproteins for each lot of IT/IF Control, a great advantage over all other commercially available positive IQC materials.
Remarkably stable (all 3 paraproteins intact over time until expiry date).
Robust performance (unless faulty analyser).

Quantitatively:
Good reproducibility & good precision
Clinically insignificant drift with time in their means (g) for the 2 lots of IT/IF Control studied.

Its potential as a full characterisation marker is therefore established.

Outcome:

Full implementation of Sebia IT/IF IQC alongside its sister Normal IQC in our routine service.
Its optimized use brings quality improvement & diagnostic assurance to our Serum Proteins Electrophoresis service:
a) ensures good detection of paraproteins in patients' blood.
b) accurate quantification of patients' paraprotein results (especially for long-term myeloma patients) down to 1 gram which is the minimum reporting threshold in this laboratory.

REFERENCES

1. I.M. BARLOW and M.L. KEMP (2010)
Evaluation of the Sebia Capillarys zone electrophoresis system for monoclonal paraprotein analysis
BRITISH JOURNAL OF BIOMEDICAL SCIENCE 2010 67 (3) 150-153
2. Sebia Capillarys & Hydrasys Technical, Training Manual PowerPoint Presentation (2019)

ACKNOWLEDGEMENTS

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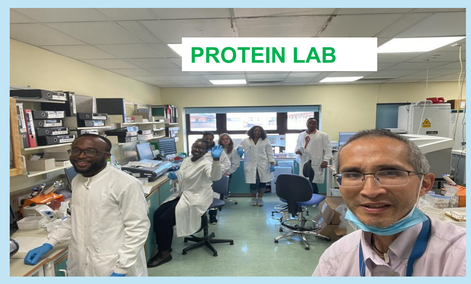

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