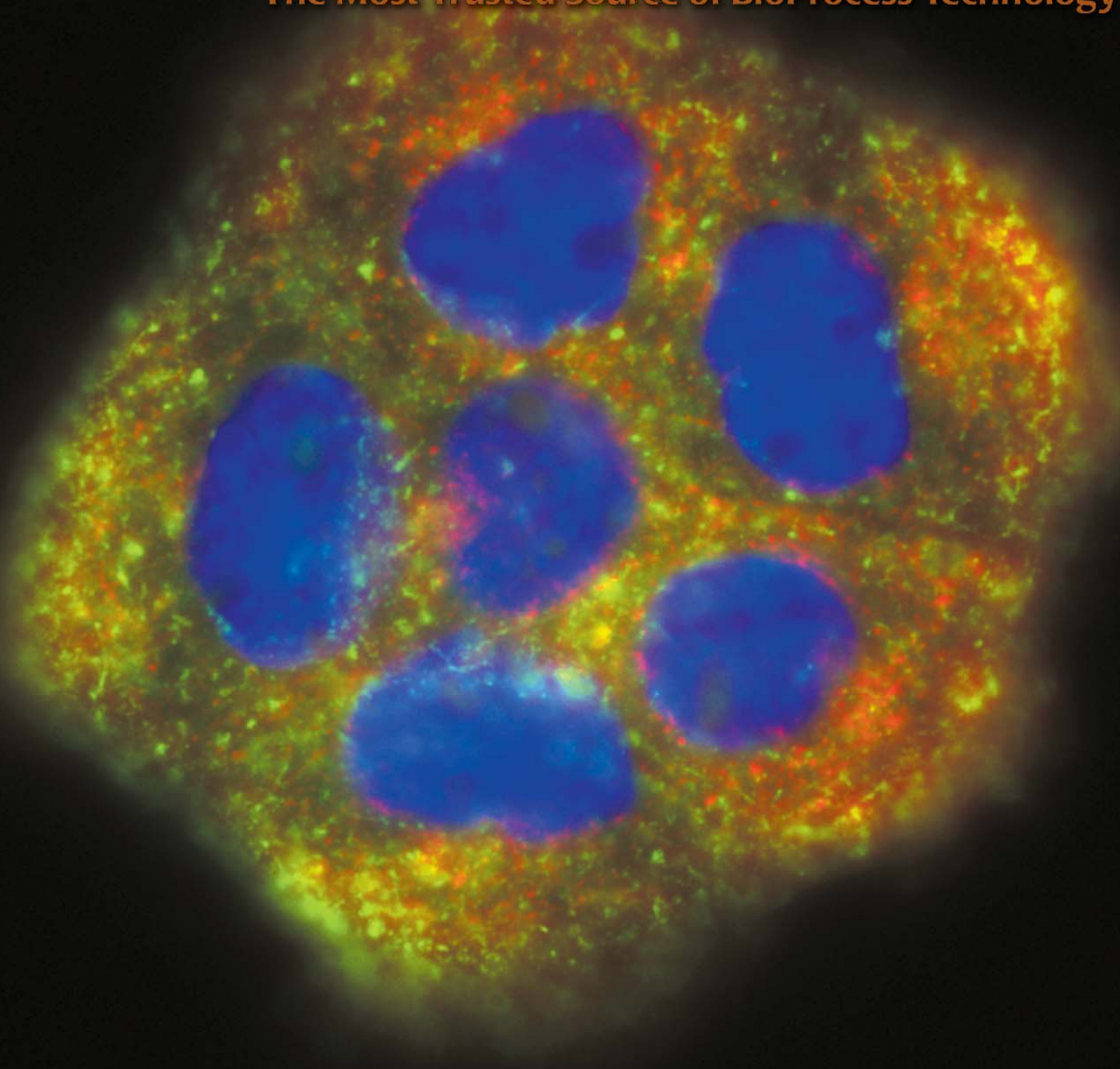


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# Rapid At-Line Antibody Titre Determination Using the MININEPH Endpoint Nephelometer

By KYM BAKER, LEANNA JONES, ALISON SMITH, HILARY HARROP, and STEVE FLATMAN

One of the major aims of modern biotechnology companies that are producing recombinant therapeutic proteins is to focus on timeline reduction of critical cell line selection and process optimisation studies in order to minimise the time and financial constraints of early development products. This “minimalist paradigm” of maximising early development throughput with minimal capital/operational outlays is a key driver for implementation of novel analytical technologies which can be applied at-line to process instrumentation.<sup>1,2</sup>

The large-scale production of recombinant therapeutics in the biopharmaceutical industry relies on in-process monitoring of product titre. Traditional titre determination methods, including enzyme-linked immunosorbent assay (ELISA) and protein A high performance liquid (immunoaffinity) chromatography (Protein A HPLC), are time consuming, and often reliant on analytical support from separate specialist teams/departments requiring detailed scientific knowledge and extensive training, with expensive capital outlay utilising large equipment.<sup>3</sup>

Such methods also are labour inten-

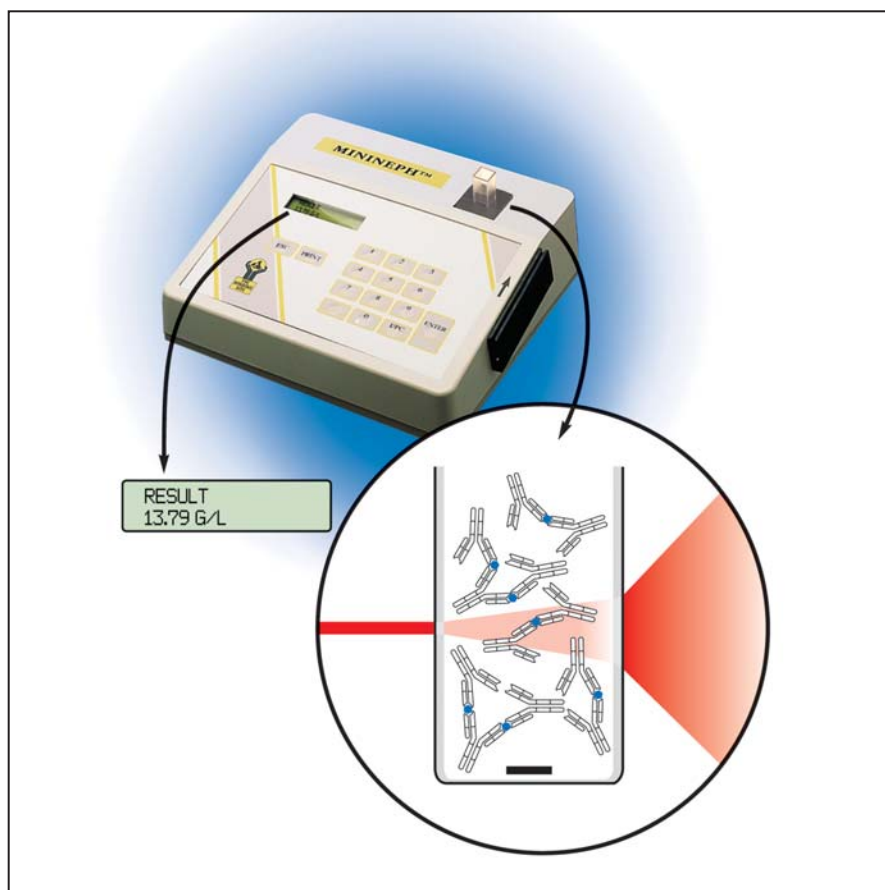


Figure 1. Schematic representation of a MININEPH assay. Laser light (red) is scattered by antigen-antibody complex formation in the reaction mixture. The degree of light scatter by immune complex formation is proportional to protein concentration (printed with permission of The Binding Site Ltd., Birmingham, UK).

sive for the analysis of a small number of samples. Using these methods, it is often uneconomical to test a single sample and so samples are typically analysed in batch mode. The subsequent waiting period (up to, and even

greater than 24 hours) for results can cause major bottlenecks and delays to critical process decisions.

In an attempt to shorten process development and optimisation timelines, new analytical technologies are

*Kym Baker, Ph.D. (kym.baker@lonza.com), is manager, process analytics; Leanna Jones is a placement student; Alison Smith, Ph.D., is senior scientist; Hilary Harrop, Ph.D., is principal scientist; and Steve Flatman, Ph.D., is department head, analytical development and quality control; Lonza Biologics, Slough, Berkshire, UK.*

being evaluated. The primary objective is to provide accurate quantitation of a recombinant product in minutes, rather than hours or days, using simple assay instrumentation applied by process personnel at-line to the ongoing process.

The ideal at-line instrument for titre determination would meet the following criteria:

- Small enough to enable titre analysis at-line to process instru-

mentation (e.g., fermenter)

- Inexpensive capital outlay, providing the option to have one instrument per process suite (or bioprocess equipment such as fermenters)
- User-friendly—easy to use with minimal training required
- Comparable or lower in cost per

sample than traditional methods

- Robust assay performance, with acceptable precision and accuracy
- High speed of analysis

The MININEPH is an instrument that fulfils all the above criteria. The MININEPH is a small endpoint nephelometer that measures specific antibody concentration in less than one minute. To date, the MININEPH has targeted the clinical market as a diagnostic tool for the measurement of proteins in human serum. In this study, we evaluated the suitability of the MININEPH to rapidly measure recombinant antibody titre produced by mammalian cell culture at-line within the fermentation process suite. We then extended the application of this process analytical technology beyond routine titre determination to monitoring other mammalian fermentation applications, including clone selection and process optimisation.

Soluble antigen in the test samples (e.g., cell culture supernatant) forms insoluble immune complexes with specific antiserum (provided in kit format). Light scattered by the immune complexes is directly proportional to the specific antigen concentration in the sample (Figure 1). The instrument is programmed with a “swipe card” calibration curve which automatically calculates the final antibody concentration without any requirements for additional hardware or software.<sup>4,5</sup>

There are a wide range of MININEPH kits available, including those specific for different antibody subclasses and other non-immunoglobulin proteins. It is important to note, however, that specific kits may not be available for many non-antibody recombinant therapeutic proteins.

Titre analysis by the MININEPH was very fast (less than one minute per reading) and was comparable in cost per sample to traditional methods. Precision was acceptable (<6% CV) and equivalent to ELISA. The instrument was easy to use and was small enough to be situated at-line to process instrumentation (e.g., next to the fermenter). In addition, the application was suitable for testing anti-

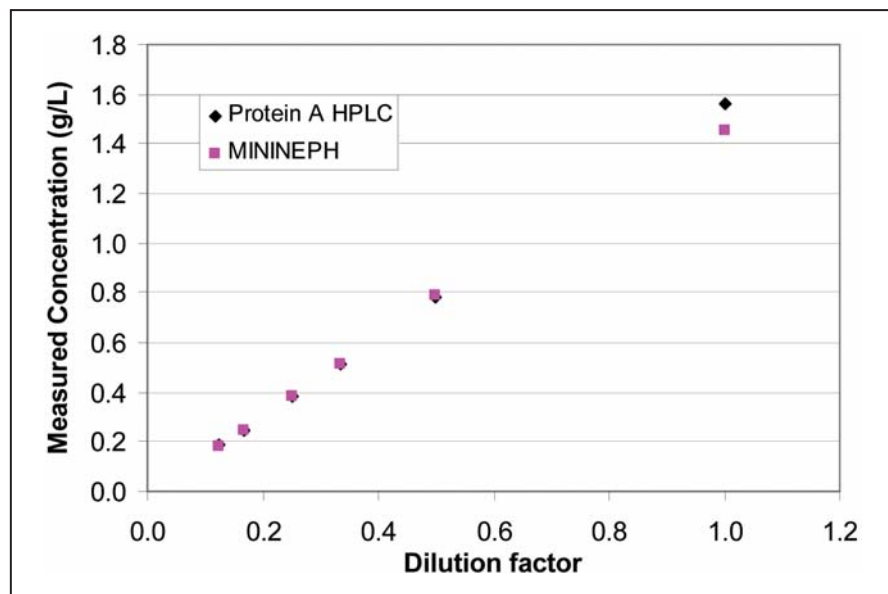


Figure 2. Plot demonstrating the correlation between MAb AM dilutions analysed by the MININEPH and Protein A HPLC.

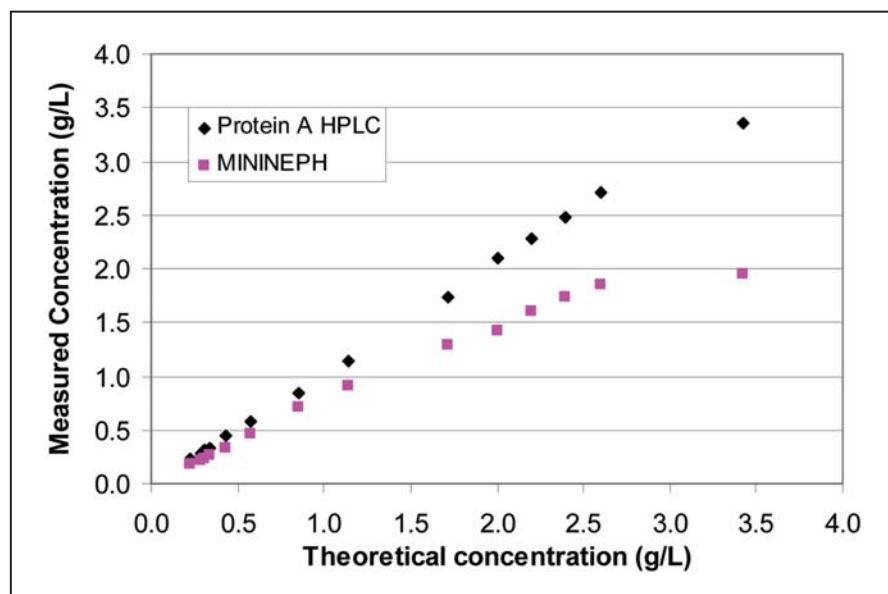


Figure 3. Plot demonstrating the correlation between MAb F dilutions analysed by the MININEPH and Protein A HPLC.

bodies of different subclasses in a range of different medium compositions and from different industrial cell lines (e.g., Chinese hamster ovary (CHO), murine myeloma (NSO)). For most antibodies tested, the working range was approximately 0.15 g/L to 2.60 g/L, making the MININEPH suitable for the direct assay of product from most industrial mammalian cell culture applications.

At Lonza, the application of the MININEPH during process development stages has enabled significant timeline reductions for model recombinant antibody projects across the life cycle of these products during both early cell culture and process optimisation stages (compared to traditional timelines). When applied generically to multiple projects, this could result in significant reductions to the overall cost of early development products, many of which may not succeed, thereby supporting minimalist paradigm requirements.

## Experimental Outline

A variety of recombinant antibodies (including an antibody fragment) were tested as part of this study. At Lonza, our generic reference method for titre determination is a Protein A HPLC quantitative immunoaffinity assay.

For each recombinant antibody evaluated, a range of different representative concentrations for that product were assayed using the MININEPH generic IgG kit (The Binding Site Ltd., Birmingham, UK) in parallel to the traditional Protein A HPLC assay. Samples were either tested neat or diluted with sample diluent (kit component). Each sample was assayed in triplicate on the MININEPH and in duplicate by Protein A HPLC to provide an estimate of intra-assay precision. In addition, the correlation between the Protein A HPLC and MININEPH mean values was determined for each product tested.

To perform an assay, reagents provided in the MININEPH generic IgG kit were used in a “magnetic card mode” containing assay calibration data specific to that kit. The swipe card also contained the information required to convert light scatter units into protein concentration units. Once loaded, this information was

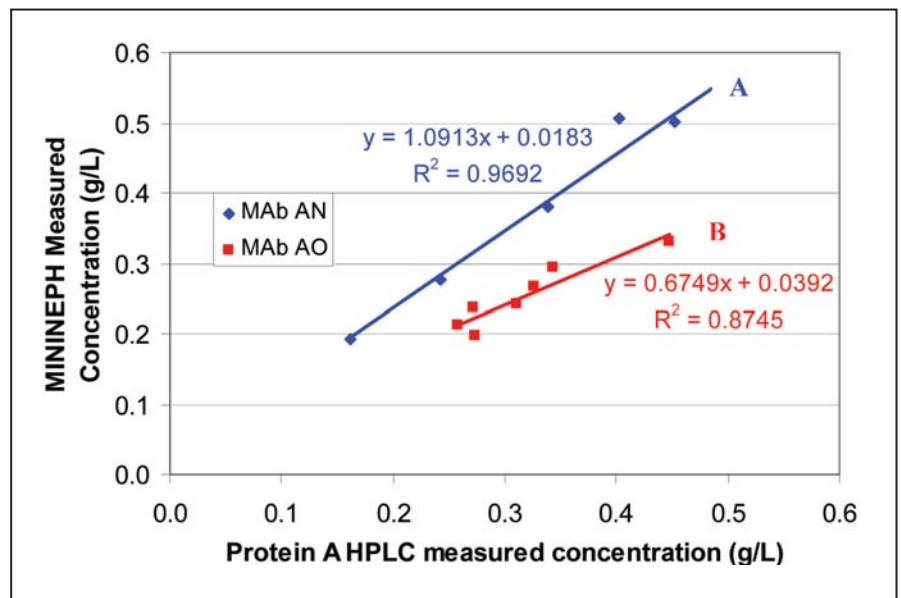


Figure 4. Plot demonstrating the linearity of different MAb assays analysed by MININEPH and Protein A HPLC: (A) GS-CHO derived MAb AN (human IgG1) and (B) GS-CHO derived MAb AO (human IgG1).

Table 1. Intra-assay and inter-assay precision for titre determination using ELISA, MININEPH, and rapid Protein A affinity chromatography.

Method	ELISA	MININEPH	Rapid Protein A Chromatography
Intra-assay precision	7 % CV	5 % CV	1 % CV
Inter-assay precision	12 % CV	6 % CV	3 % CV

stored in the MININEPH for future use with the same lot of reagents. As these parameters were antiserum lot-specific, each lot of kits contained a specific calibration swipe card.

A test sample (15 µL) was added to the bottom of a disposable cuvette containing a disposable magnetic stirring bar (provided in kit). The sample identification details and dilution factor details were entered onto the MININEPH keypad and the cuvette then placed into the reaction chamber of the MININEPH. An electronic pipette was used to add 400 µL of reaction buffer (kit) and 40 µL of specific antisera (kit). After these additions, the assay is automatically initiated. The MININEPH-specific pipette permits two different solutions to be aspirated in the same pipette tip, separated by an air gap and then

dispensed together with an automatic blow-out. This improves assay precision by eliminating operator variation due to differences in timing of the assay start. The electronic pipette was also used to achieve accurate dilutions by aspirating test sample and sample diluent.

A background “time zero” scatter reading was taken for each assay at the beginning of the antibody/antigen reaction, and then a second reading was taken after, for example, 15 seconds (generic IgG kit). The MININEPH uses the difference between these two readings to calculate protein concentration, taking any dilution factor into account, and results are reported and printed (in g/L).

## Results and Discussion

A wide variety of recombinant antibody-

ies were found to be suitable for use with the MININEPH generic IgG kit:

- Recombinant antibodies derived from both industrial GS-NSO and GS-CHO cell lines
- Human IgG<sub>1</sub>, IgG<sub>2</sub>, and IgG<sub>4</sub> subclasses, including a chimeric IgG<sub>4</sub>
- Antibodies comprising either kappa or lambda light chains
- An antibody fragment
- Recombinant antibodies produced in a range of media from chemically defined animal-component-free (CDACF) through to complex protein-containing media

The dynamic working range for each antibody was estimated from linear regression analysis of a plot of the measured antibody concentration by Protein A HPLC against the measured antibody concentration following MININEPH analysis. If the MININEPH determined that concentrations for a particular antibody were consistently lower or higher than the reference Protein A HPLC method, then a specific correction factor was required to convert the MININEPH values into Protein A HPLC equivalent values.

It was assumed that if there was less than 10% variability between MININEPH and Protein A HPLC values, then no correction factor was required. For the majority of recombinant antibodies tested, there was a strong correlation between the Protein A HPLC and MININEPH measurements.

## Case Studies

### Case Study One: MAb AM

MAb AM was a human IgG<sub>1</sub> produced in CDACF media by a GS-NSO cell line. A MAb AM sample was diluted to provide different concentrations ranging from 0.16 g/L to 1.57 g/L.

For the concentrations tested, MAb AM demonstrated a linear correlation with <10% difference between MININEPH and Protein A HPLC values over a range of 0.19 g/L to 1.56 g/L, and no correction factor was required to “convert” MININEPH values to Protein A HPLC values (Figure 2).

### Case Study Two: MAb F

MAb F was a chimeric IgG<sub>4</sub> produced in CDACF media by a GS-NSO cell line. An MAb F sample was diluted to provide different concentrations ranging from 0.21 g/L to 3.43 g/L.

The MAb F antibody Protein A HPLC and MININEPH results did not demonstrate a correlation close to 1 and a correction factor was required to convert the MININEPH data into Protein A HPLC comparable values (Figure 3). For each MAb F sample, the MININEPH generic IgG kit consistently measured lower concentrations than Protein A HPLC (>10 % difference). In addition, the MININEPH demonstrated a reduced working range compared to the reference Protein A HPLC method over which the titre measurement was valid. Nevertheless, this antibody still demonstrated a ten-fold dynamic range (0.23 g/L to 2.60 g/L), based on the concentrations tested using the MININEPH instrument.

This case study, however, highlights the need to evaluate the working dynamic range for each antibody product specifically for the MININEPH method prior to routine use, as these may differ from the reference titre method. In addition, the application of a calculated correction factor to the MININEPH data falling within the working range of the assay is required to convert the MININEPH data-to-data comparable to reference method titres.

### Case Study Three: MAb AN and MAb AO

The correlation between Protein A HPLC and MININEPH measurements

Table 2. Capital and running costs per sample for titre determination methods.

Method	Instrument* (US \$1000's)	Sample (US \$)
Rapid Protein A chromatography	100	1.8
ELISA	10	1.8
MININEPH	10	1.8

\* Includes IT hardware and software

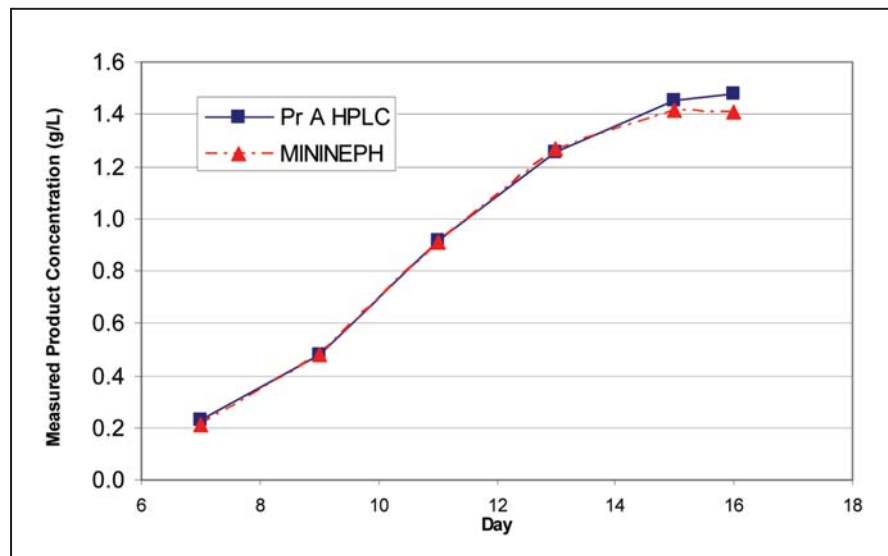


Figure 5. Daily at-line monitoring of MAb AG titre throughout a fermentation using the MININEPH.

was antibody-specific and independent of subclass, industrial cell line (GS-CHO or GS-NSO) used to manufacture it, and medium composition (Figure 4). Figure 4 demonstrates the differences in titre measurement for two human IgG<sub>1</sub> antibodies, MAb AN and MAb AO, produced in GS-CHO cells. The MININEPH measured slightly higher concentrations than Protein A HPLC for MAb AN (but within 10%) but lower concentrations for MAb AO. A correction factor was required to convert the MAb AO values to Protein A HPLC equivalent values.

### Precision of Measurement

The intra-assay and inter-assay precision of titre determination using the MININEPH precision were estimated and compared favourably to the precision of traditional methods such as ELISA, making it an ideal tool for early process development monitoring (Table 1). The precision of the validated Protein A HPLC assay, however, demonstrated why the method remains the method of choice at Lonza for concentration determination at critical manufacturing process stages (e.g., purification column loading) to a Good Manufacturing Practice (GMP) standard. Therefore, the use of the MININEPH at Lonza is currently restricted to process development applications for which lower precision (approximately 6% CV) can be tolerated.

### Ease of Use

We have compared the MININEPH procedure against ELISA and Protein A HPLC methodologies. ELISA is a relatively labour-intensive method to undertake, requiring multiple dilutions and, generally, a much higher level of training and expertise to perform and interpret the data. In contrast, many of the process samples evaluated in this study could be tested neat using the MININEPH, thus eliminating dilution errors that are common with traditional methods such as the ELISA. In addition, any necessary dilutions could be performed with the MININEPH electronic pipette for improved accuracy.

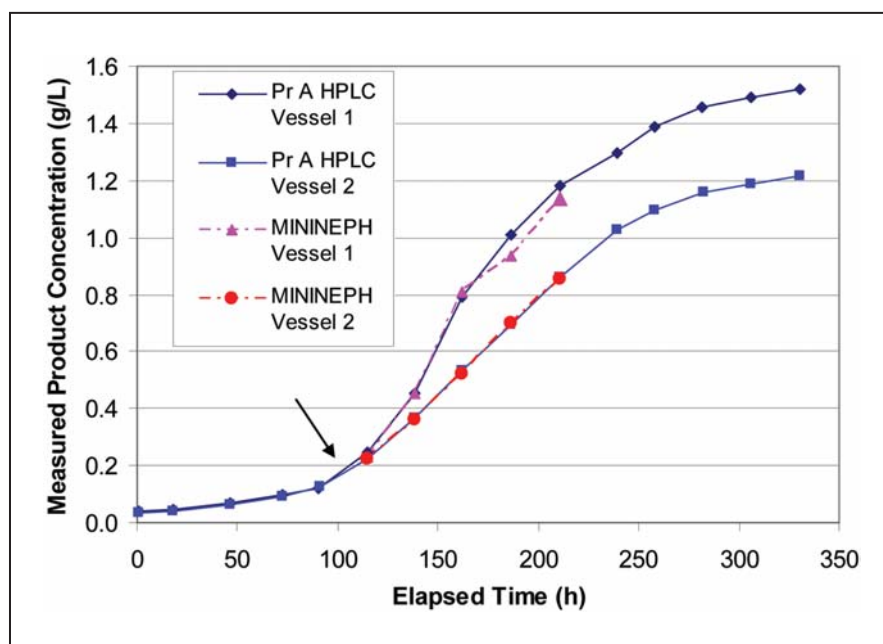


Figure 6. Use of the MININEPH to monitor fermentation optimisation studies (temperature). MAb AM cultured in two different fermenters (vessel 1 and vessel 2). The arrow indicates when the temperature was decreased in vessel 2 but not vessel 1.

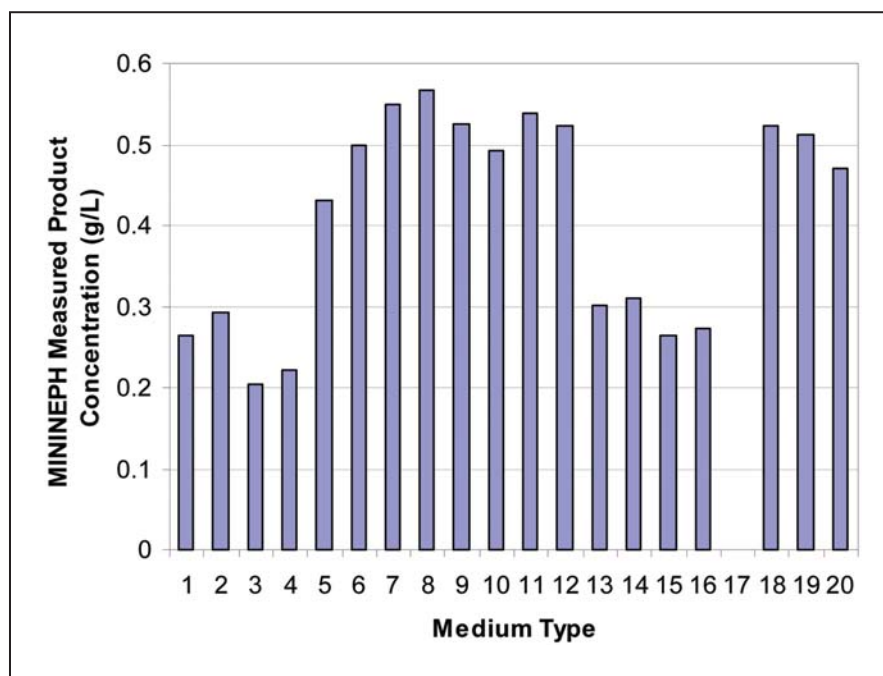


Figure 7. Media optimisation analysis using the MININEPH for MAb F.

Although the price per sample is comparable for each of the methods compared herein, the capital outlay for the MININEPH is considerably lower than the HPLC-based rapid affinity chromatography method, but comparable to ELISA equipment (Table 2).

Performing a MININEPH assay was extremely fast and easy, and new operators were routinely fully trained with minimal effort (i.e., <1 day). Additionally, with each assay taking less than one minute, the MININEPH can economically test single samples to add

flexibility to timelines and testing. This is not readily applicable to the ELISA or Protein A HPLC methods.

### Process Monitoring Applications of the MININEPH

The potential benefits of using the MININEPH for process monitoring during, for example, mammalian cell culture are considerable. It is a small, economical, and easy to use instrument, making the MININEPH an ideal tool for routine monitoring and troubleshooting of recombinant protein production. The MININEPH is small enough to be situated conveniently next to the fermenter (e.g., "at-line") and the low capital outlay justifies having multiple instruments in a process suite, negating the requirement for batch testing by separate analytical teams/departments.

The MININEPH has been applied to multiple applications throughout the life cycle of antibody products at Lonza, including: 1) clone selection; 2) process optimisation studies; and 3) titre monitoring (multiple applications).

### Titre Monitoring

The MININEPH is ideal for at-line monitoring of antibody production on a daily basis (or more regularly, as desired) (Figure 5). For the MAb shown in Figure 5, the MININEPH results demonstrated a good correlation to the Protein A HPLC data. However, the placement of a MININEPH instrument at-line to the fermenter permits daily titre determinations in less than one minute, whereas Protein A HPLC data are routinely batch tested. The MININEPH allows the progress of the fermenter to be monitored so any problems can be identified immediately.

### Cell Culture Process Optimisation

#### A) Temperature Shift Optimisation

The MININEPH was used to monitor process optimisation studies including temperature shift and media optimisation experiments. In the temperature shift experiment, cells producing MAb AM were grown in two different fermenters maintained either at the

routine temperature or decreased to a lower temperature after a set time point (Figure 6). The decrease in temperature in vessel 2 resulted in production of MAb AM decreasing significantly in

vessel 2 compared to vessel 1. Lonza did not have the MININEPH implemented at the time of this experiment, but retrospective analysis of these samples demonstrated that by using the MININEPH,

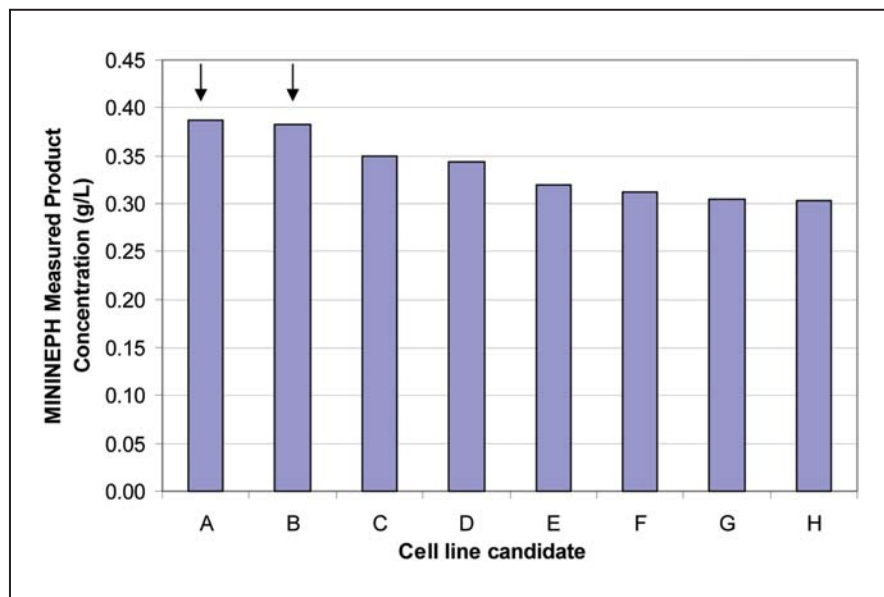


Figure 8. Using the MININEPH for clonal selection (MAb F). The arrows indicate the two clones chosen for progression in less than 10 minutes.

Table 3. Advantages and disadvantages of the MININEPH.

Advantages	Disadvantages
Very fast (<1 minute per reading)	May not be suitable samples with low protein concentration (e.g., <0.15 g/L; early development samples)
Good correlation between MININEPH and Protein A HPLC values	
Majority of samples can be tested neat negating a requirement for dilution	MININEPH values may need to be converted to Protein A HPLC values
Working range: ~ 0.15 g/L to 2.6 g/L	
Small sized instrument (23.3 cm wide x 23 cm long x 8.5 cm high)	MININEPH inter-assay precision (6% CV) inferior to reference method Protein A HPLC (3% CV)
Inexpensive capital outlay	
Easy to use (minimal training required)	Available kits restricted primarily to antibody targets
Low cost per sample (<US \$2)	
MININEPH inter-assay precision (6% CV) superior to ELISA (12% CV)	

the fermentation operator could have identified that the lower temperature was detrimental to MAb production at a significantly earlier stage and therefore, could have ceased culturing vessel 2 up to nine days sooner.

### **B) Media Optimisation**

Media composition analysis was optimized using the MININEPH. In this study, cells producing MAb F were grown in twenty different media permutations to determine which concentration of three separate variables resulted in the best product titre (Figure 7). Further fermentations were dependent on the assay results, so rapid turnaround of titre results was important for this investigation. The MININEPH, therefore, was the method of choice for monitoring product concentration. Each sample was tested in triplicate and the mean, standard deviation, and coefficient of variation (% CV) determined for each sample. Medium compositions were compared against control medium conditions (fermentations 9, 10, 11, 12, 19, and 20; Figure 7). As demonstrated in Figure 7, none of the medium changes demonstrated any statistically significant improvements compared to the control cultures, although the three leading variations were further evaluated (7, 8, and 18). Medium condition 17 gave values below the limit of quantitation for the MININEPH (<0.16 g/L).

### **Clone Selection**

The MININEPH has also been used during clonal selection stages to select the highest producing cell lines for scale-up prior to fermentation evaluation in minutes, rather than waiting hours or days for ELISA or Protein A HPLC results, enabling process development scientists to make decisions “at line” to their fermentations (Figure 8). The correction factor for MAb F had previously been determined (1.33). Over the working range for this MAb (0.2 to 2.6 g/L) the MININEPH consistently demonstrated acceptable precision (<4% CV). Therefore, clones A and B were statistically determined to be significantly higher producers than the remaining

clones (at a 95% confidence level).

It is worth noting that the MININEPH may not be suitable for very low producing cell line processes as the instrument is currently only appropriate for measuring  $\geq 0.15$  g/L. The optimised processes used at Lonza for MAb F have been very successful. This specific recombinant antibody process has led to the selection of a cell line producing 5.5 g/L. Lonza is currently investigating innovative technologies to enable the use of the MININEPH at earlier stages of the cell line selection process specifically aimed at targeting lower concentration ranges (<0.15 g/L).

### **Advantages and Disadvantages of the MININEPH**

The advantages and disadvantages of the MININEPH are summarised in Table 3. The MININEPH has been demonstrated to fulfill some of the key drivers for supporting the minimalist paradigms of development projects in biotechnology industries: primarily the reduction in both cost and elapsed time, and also the empowerment of process analysts to control processes at-line by removing the bottlenecks associated with the dependency on other teams/departments (typical of medium-to-large biotechnology organisations).

In addition, at-line titre determination also enables faster progression of innovation projects (e.g., medium optimisation) and improved utilisation of process capacity as suboptimal processes (e.g., fermenters) can be terminated earlier, facilitating turnaround of vessels for subsequent process evaluations. And although outside the scope of the current study, this may be especially pertinent to particularly rapid fermentation processes such as bacterial and/or yeast cell-based processes for which there is a more immediate requirement to rapidly assess data to determine harvest criteria.

### **Conclusions**

In conclusion, the MININEPH provides an ideal development tool for process analysts and is capable of measuring antibody titre from various processes including (but not limited to) cell culture

supernatants. This compact instrument enables process operators to be self-sufficient and reduces the dependency on specialised analytical departments, thereby reducing development timelines. The MININEPH is suitable for testing a wide range of antibodies (and potentially other products), regardless of antibody subclass or cell line used. There is usually good correlation between MININEPH and traditional reference method results (Protein A HPLC).

The MININEPH has improved the efficiency of mammalian cell fermentation process development at Lonza Biologics. Development timelines are now less dependent on traditional analyses and can progress faster and more efficiently. Timelines are constantly being reduced as the bottleneck of waiting for assay results from a separate analytical department has decreased. In return, specialist analytical personnel have fewer requests for daily process titre testing of small numbers of samples and can also spend more of their time on more complex analytical problems.

Following the potential development of innovative MININEPH assays to provide improved sensitivity at Lonza, we anticipate further timeline reductions in the years to come.

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