Effective transfer of *in vivo* monoclonal antibody production to a highly efficient and economical *in vitro* method

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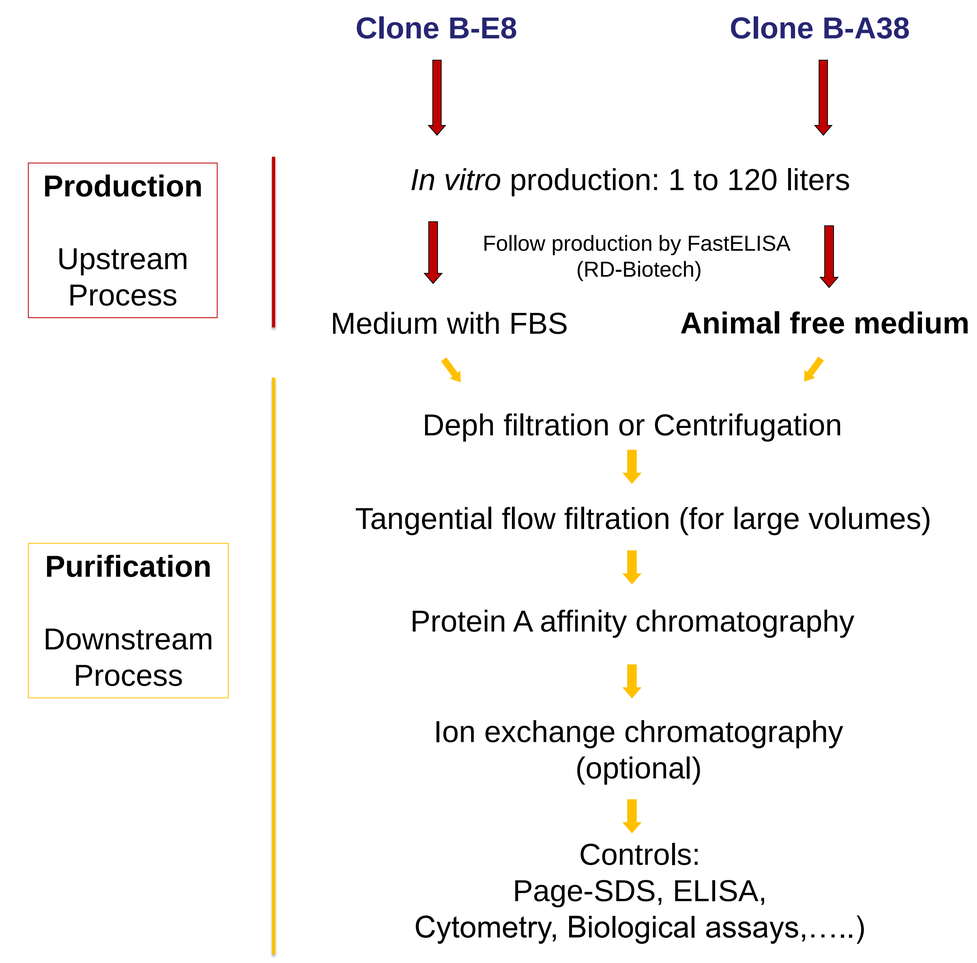
Introduction

The production of monoclonal antibodies (mAbs) using the ascites method is becoming increasingly more difficult to justify due to the pain and distress caused to the host animal and the availability of alternative in vitro methods. RD-Biotech, a major provider of bio production services in France, has developed and optimised an economical approach for *in vitro* production of mAbs.

The work presented here illustrates the successful production and purification of two mAbs using this methodology. We have been able to compensate low mAb concentration in some culture supernatants by significantly increasing the volume of production up to 120L per batch in totally serum free medium, thus enabling high yields.
The rapid production phase or upstream process (around 12 days) was demonstrated to avoid any instability problems occurring with some hybridoma. Downstream and purification followed a GMP like process as follows: Clarification of culture supernatant was performed with deep filtration filter unit followed by a tangential flow filtration step in order to concentrate by a factor of 15. Then monoclonal antibodies were purified by protein A affinity chromatography followed by ion exchange chromatography as a polishing step. We show in the present study that antibodies produced by our technology have the same properties as the reference *in vivo* produced antibodies.

This cost effective, rapid *in vitro* technology yields highly purified antibodies (from few mg up to several grams) and represents a highly competitive alternative technology to hollow fiber technology which remains time consuming, very expensive and less adaptable to some applications.

Material & Methods



Results

Clone B-E8

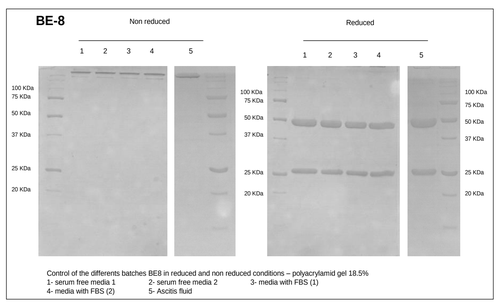
Relative expression of the B-E8 mAb in different media

Sample N°	Origin	yield of production (% ref)
1	animal free medium	24%
2	animal free medium	15%
3	medium containing FBS (1)	133%
4 (ref)	medium containing FBS (ref)	100%

(% of the reference, measured with FastELISA kit from RD-Biotech (Code rdb-3256)

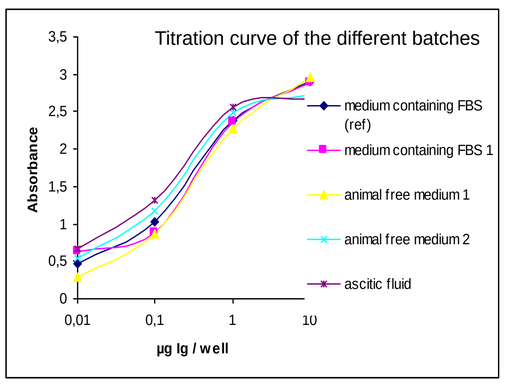
↳Production yield: More important in media with FBS

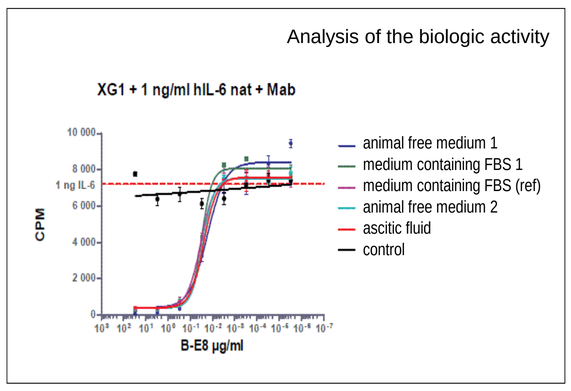
PAGE-SDS analysis



↳ mAb purity: Excellent in all conditions

Characterisation of the purified mAb





Blocking IL-6 activity evaluated in-vitro by a proliferation test: inhibition of XG1 growth in presence of IL-6 and of increasing B-E8 Mab concentration.

↳ Same titers
↳ Same Biologic Activity → **mAbs Quality: EXCELLENT**

Clone B-A38

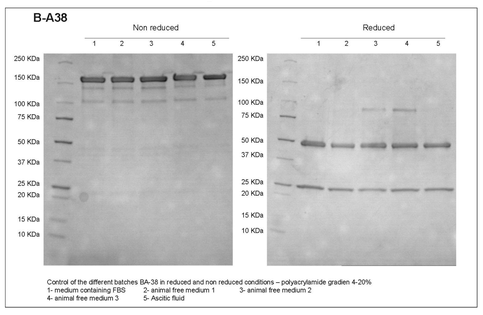
Relative expression of the B-A38 mAb in different media

Sample N°	Origin	yield of production (%)
1 (ref)	medium containing FBS (ref)	100%
2	animal free medium 1	195%
3	animal free medium 2	95%
4	animal free medium 3	94%

(% of the reference, measured with FastELISA kit from RD-Biotech (Code rdb-3256)

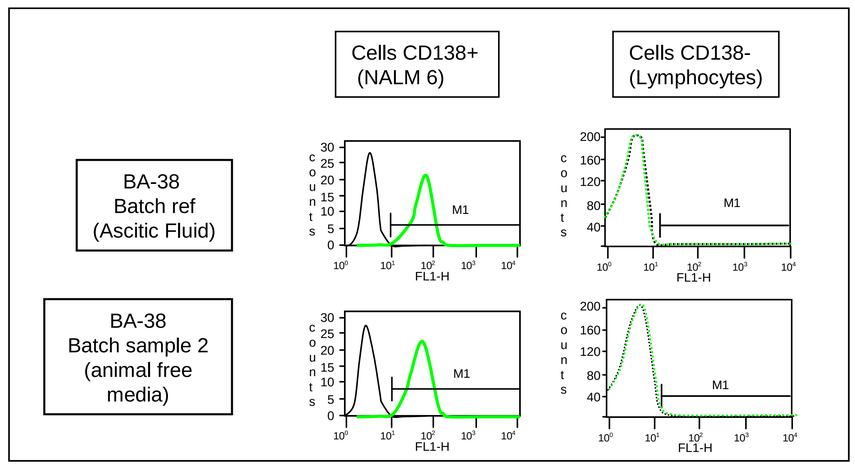
↳Productivity: Excellent in Free animal production environment

PAGE-SDS analysis



↳ mAb purity: Excellent in all conditions

Specificity study of the purified mAb



Same profile for all batches

↳ Specifity of the *In vitro* produced mAbs: EXCELLENT

Conclusions

We demonstrate here that transfer for *In vivo* production of mAb to *In vitro* production can be achieved in relatively good conditions and with an equivalent quality of the purified antibodies. Although our technology requests high volumes of supernatant in order to reach desired quantities of antibodies, it is highly competitive to other techniques using hollow fibers system or permeable system with separated cell compartment. Our technology is much faster; 100 Liters may be produced within 12 days of culture (up to 15 grams of MAbs) instead of several weeks or months for other techniques.
Although downstream may need (depending of clone) depth filtration and tangential flow filtration, cost of goods are far cheaper and can effectively compete with *in vivo* production.
The technology has already been validated for more than 150 different hybridoma's. In addition it has been proven to work successfully with antibodies of different origins, including rat and recombinant humanized antibodies.

For more informations, please visit our website **www.rd-biotech.com.**