ANALYSIS OF ALTERNATIVES

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SOCIO-ECONOMIC ANALYSIS

Legal name of Applicant(s):	SEBIA
Submitted by:	SEBIA
Substance:	4-(1,1,3,3-Tetramethylbutyl) phenol, ethoxylated [covering well-defined substances and UVCB substances, polymers and homologues]
Use title:	Use-1 Industrial use of 4-tert-OPnEO for its "wetting" detergent properties in the production of buffers, reagents and gel supports allowing the dissolution, the dilution and the good spreading of substrates and reagents, necessary to optimize the functioning and the sensitivity of gel electrophoresis *in vitro* diagnostic tests
Use number:	1

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LIST OF ABBREVIATIONS

4-tert-OPnEO	4-(1,1,3,3-Tetramethylbutyl)phenol, ethoxylated [covering well-defined substances and UVCB substances, polymers and homologues]
AfA	Application for Authorisation
AOX	Adsorbable Organic Halogen
AP	Alkaline Phosphatase
B	Billion (€)
CEA	Cost-effectiveness analysis
COD	Chemical Oxygen Demand
CSF	Cerebro-Spinal Fluid
CV	Coefficient of Variation
CZE	Capillary Zone Electrophoresis
DNA	Deoxyribonucleic Acid
FDA	Food and Drug Administration
HLB	Hydrophilic-Lipophilic Balance
IEF	Isoelectrofocusing
IVD	*In Vitro* Diagnostic
k	Thousands (€)
M	Million (€)
NPnEO	N-phenol ethoxylated
NPV	Negative Predictive Value
PPV	Positive Predictive Value
PV	Present value
SD	Standard Deviation
WHO	World Health Organisation

1. SUMMARY

CONTEXT

SEBIA is an *in vitro* diagnosis kits manufacturer here filing an authorisation for its use of 4-(1,1,3,3-Tetramethylbutyl) phenol, ethoxylated compounds in the production of electrophoresis reagents and devices. Indeed, under Use-1, SEBIA offers high performance solutions intended in the determination of disease markers and subject to strong regulatory and quality requirements, such as the one encountered in medical and clinical sectors. Under Use-1, SEBIA uses pure solutions or mixtures of 4-tert-OPnEO in the formulation of tests reagents and buffer solutions, so as to meet normative and customer requirements, in particular in terms of sensitivity, specificity, reproducibility and accuracy. Moreover, to these reagents and kits are associated instruments dedicated to the identification and the quantification of specific proteins in a given sample. The global system thus allows the interpretation of biological results relative to the screening, the follow-up and the prognosis of various diseases linked to haemoglobin and protein abnormalities.

Use-1, and the level of performance provided by 4-tert-OPnEO-based substances, is an essential condition for the competitiveness of SEBIA; its banning will impact a major share of its activity and could jeopardize its survival.

SUBSTANCE FUNCTION

In order to maintain their proper functions (mild detergency, wettability, proteins stabilization linked to non-ionic properties) throughout their use in biological and medical applications, two main requirements apply in the design of reagents concerned by Use-1: clinical and analytical performances. Each of these characteristics is further detailed in the following subsections. In what follows, it has been identified some detergents which, regarding the kit concerned and the reagent involved in the analysis reaction, could provide suitable properties for electrophoresis kits: as an example, Tween® 20 could present interesting properties suitable to expected functions, according to recent works carried out on other product references. Nevertheless, SEBIA will have to pursue its R&D initiative to identify the best alternative solution.

IDENTIFICATION OF ALTERNATIVES

A significant work of research is currently carried out by SEBIA to identify several potential alternatives. These alternatives are being assessed for technical, normative and customer functional requirements which justifies the review period requested in the context of the current Application for Authorisation. Indeed, in addition to the difficulty of identifying and developing a technically and economically viable alternative, substantial internal and external validation processes have been considered in the calculation of the expected timeline.

"APPLIED FOR USE" AND "NON-USE" SCENARII

Under the "applied for use" scenario, SEBIA will pursue the use of 4-tert-OPnEO for its detergent properties ensuring the dilution, the dissolution and the good spreading of reagents and solutions, necessary for the results interpretation of IVD electrophoresis

tests based on protein separation, during the period of time necessary to develop, implement and validate an alternative process, thereby securing both its activity and the supply of chromatography tests to clinical laboratories and hospitals.

So, taking into account the high level of requirement associated with Use-1, its importance for SEBIA in terms of business, know-how and competitiveness, the most likely non-use scenario is, with the interdiction of 4-tert-OPnEO, the cease of manufacturing of 133 gel electrophoresis assays, i.e. all the gel references commercialized by SEBIA. So, since Use-1 concerns an important part of SEBIA's current and potentially future portfolios, this scenario will have therefore strong economic, social and wider impacts on SEBIA Company and the Society in general.

IMPACTS OF GRANTING AUTHORISATION

Regarding the endocrine disrupting properties of the degradation product of the substances concerned by this AfA, the "applied for use" scenario describes how the production activities of SEBIA linked to the use of the substance can contribute to the current chemical and ecological status of the catchment area where are located SEBIA facilities. Indeed, phenol ethoxylated substances are now subject to an increased surveillance because of their potential environmental toxicity. However, in the immediate environment of production activities linked to the use of the substance by SEBIA described in this dossier, 4-tert-OPnEO compounds were measured in very low quantities contrasting with the relatively numerous activities potentially releasing octylphenols in the catchment area.

So, as a realistic hypothesis for the "applied for use" scenario, it can be considered that discharges generated by SEBIA activities are insignificant in the current situation and will remain so, throughout the requested review period.

HYDRAGEL® products concerned by Use-1 represent around 30% of the global Company's turnover. **Under Use-1 are included all the references of electrophoresis gel manufactured and commercialized by SEBIA. The ban on using the substance will thus lead to the cease of production of the range and more extensively, to the bankruptcy of the Company. It would thus result in the definite cessation of its activities and consequently, in the layoff of all the employees of SEBIA and its subsidiaries. So, in this context, the total monetised impacts of the "non-use" scenario amount to € 1.5B.**

So, based upon a qualitative appreciation of ecological and eco-toxicological impacts, and a quantitative evaluation of business and social issues, the socio-economic benefits outweigh the risks arising from the use of the substance.

CONCLUSION

Based on the argument put forward, SEBIA requests, for activities concerned by Use-1, a twelve-year review period in order to develop, implement and qualify an alternative solution.

2. AIMS AND SCOPE OF THE ANALYSIS

SEBIA is a downstream user of 4-(1,1,3,3-Tetramethylbutyl) phenol, ethoxylated (4-tert-OPnEO) employed as a surfactant in the production of *in vitro* diagnosis kits based on electrophoresis methods. Under Use-1, 4-tert-OPnEO is used as one commercial solution (Triton™ X-100) at two sites in France for the production of electrophoresis-based assays.

4-tert-OPnEO provide key properties in the production of *in vitro* diagnosis kits, for which alternatives have yet to be identified, developed, validated, industrialised and registered by Regulatory Authorities of each country where the devices are sold.

It is used in the production of several products of SEBIA's portfolio that constitute the core of the company's offer. A ban on its use would generate significant in terms of loss of revenues and profits, as well as loss of employment and significant external impacts on patients health and healthcare systems.

The aim of the present document is to provide a comprehensive analysis of both the Analysis of Alternatives and Socio-Economic Analysis parts of SEBIA's Application for Authorisation of Use-1, i.e.:

- to provide a comprehensive understanding of the context of the AfA;
- to describe SEBIA's initiatives of research for alternatives, potential alternatives and substitution strategy;
- to provide a cost-effectiveness assessment of the application.

For the sake of clarity, it is reminded that this document is part of a broader AfA as SEBIA's application comprises four uses:

Use-1	**Industrial use of 4-tert-OPnEO for its "wetting" detergent properties in the production of buffers, reagents and gel supports allowing the dissolution, the dilution and the good spreading of substrates and reagents, necessary to optimize the functioning and the sensitivity of gel electrophoresis *in vitro* diagnostic tests**
Use-2	Industrial use of 4-tert-OPnEO for its detergent properties in the production of electrophoresis gels in view of ensuring the positioning of specific proteins necessary for the interpretation of results of gel electrophoresis *in vitro* diagnostic tests
Use-3	Industrial use of 4-tert-OPnEO for its detergent properties resulting in cellular lysis and protein interactions rupture and required for the production of reagents involved in the determination of proteins of interest in gel and capillary electrophoresis IVD tests
Use-4	Industrial use of 4-NPnEO for its detergent properties in the production of buffers and reagents in view of ensuring the positioning of specific proteins necessary for the interpretation of gel electrophoresis *in vitro* diagnostic tests results based on the determination of isoenzymes

Table 1. Uses of the Application for Authorisation
Note: Other uses are detailed in separate documents

2.1. SEBIA

SEBIA was founded by Guy Barouh in 1967 and developed rapidly with the introduction of a reagent based on cellulose acetate. The company has had a significant impact in the field electrophoresis reagents replacing the paper by cellulose acetate, making it possible to obtain results in one hour (as against almost 12 hours using paper) while improving the reliability and accuracy of the analysis.

This technological breakthrough was accompanied by the development of devices for interpreting results. In 1971, SEBIA launched the CELLOMATIC® equipment, first fully electronic integrating densitometer that enabled one to quantify results and print them in graphical form. In 1979, SEBIA created the CELLOSYSTEM® the first densitometer that incorporated a microprocessor.

Reagents based on cellulose acetate dominated the market for electrophoresis until 1986, when SEBIA introduced HYDRAGEL® reagents based on agarose gel. Gel reagents are ready-to-use, and much more sensitive than cellulose acetate-based reagents.

In 1993, SEBIA launches the HYDRASYS® equipment, one of the first systems able to carry out semi-automatically all types of electrophoresis on agarose gels. This opens the way to a maximum standardisation for handling operations and considerably reduced the risk of error.

The third technological breakthrough that marked the development of SEBIA is the launch of the capillary technique and the introduction of the CAPILLARYS® system in 2001, a new generation capillary electrophoresis system allowing complete automation of the technique, from primary sample tube to final result. With its integrated bar code reading, it ensures full traceability of the samples.

Since 2001, the Company's development has been particularly strong in the field of capillary electrophoresis with introduction of new tests including haemoglobin, immunotyping, quantification of the CDT (detection and monitoring of the alcoholism) and quantification of the HbA1c.

Led by Jean-Marc Chermette from 2017, SEBIA has seen its results grow by 8% per year. Since 1967, the company has forged an international leadership on disease diagnosis. Based on electrophoresis technology, proteins can be analysed in order to screen, to diagnose and monitor various diseases and physio-pathological conditions; primarily oncology (multiple myeloma), metabolic disorders such as diabetes and also haemoglobinopathies and rare pathologies.

Located in Lisses, in the south of Paris in France, SEBIA headquarters integrate all primary functions, such as R&D, manufacturing, customer services, marketing and commercial activities. In this way, SEBIA manages the entire reagent, software and instruments cycle: from conception to commercialization, through development and production. If the production is exclusively located in France, SEBIA covers directly or through subsidiaries (Benelux, UK, Italy, Spain, Germany, USA…), representative offices (China, Brazil and Doha) or through a network of distributors, more than 120 countries in the world. Consequently, thanks to its international development, the company now generated nearly 80% of its turnover in export.

SEBIA Group has almost 580 employees worldwide, 50% of which are based in France, where 45 members are dedicated to R&D. Since 1967, SEBIA developed new electrophoresis solutions and systems for the clinical laboratories by bringing new diagnostics tools through better automation throughput and improved analysis accuracy (***Fig. 1***).

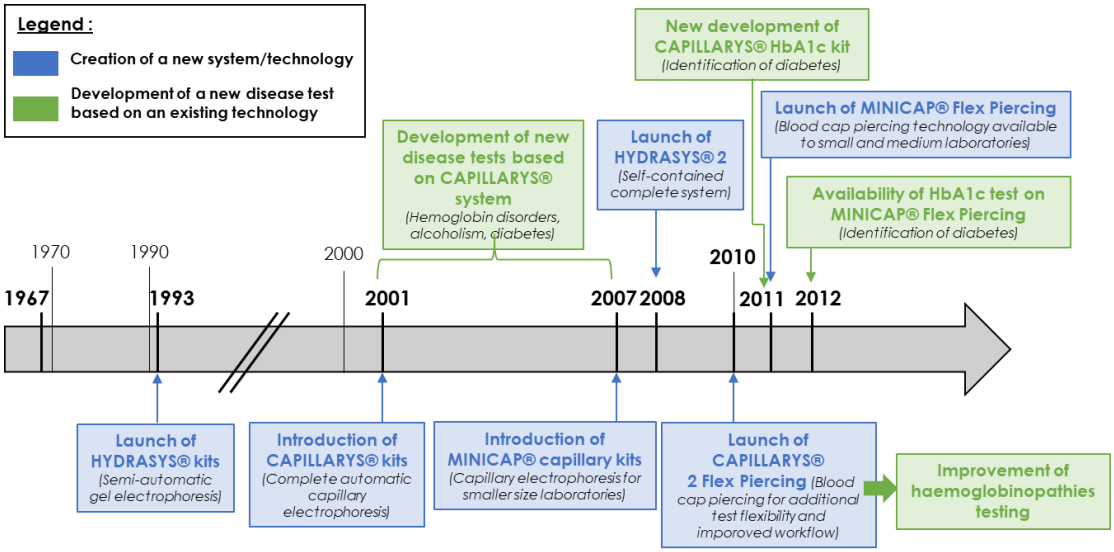


Figure 1. Main products developed by SEBIA since its foundation

Presently, this team is especially focused on new solutions to unmet needs in key pathologies like HbA1c quantification, haemoglobin adult or neonatal testing, but also in less prevalent diseases such as α-1-Antitrypsin deficiency or immunological characterization of IgG oligoclonal banding for suspected cerebrospinal fluids or detection of β-2-transferrin in flow liquid. To date, SEBIA's portfolio comprises over 35 kinds of gel and capillary electrophoresis tests, 26 of which contain 4-tert-OPnEO and 4-NPnEO; more than 8000 capillary instruments were sold worldwide in 2015.

2.2. Scope of the AfA

4-(1,1,3,3-Tetramethylbutyl) phenol, ethoxylated [covering well-defined substances and UVCB substances, polymers and homologues], referred to as 4-tert-OPnEO in the present document, is classified under REACh as a Substance of Very High Concern due to Endocrine disrupting properties for the environment (according to Art. 57(f)) of its degradation product, 1,1,3,3-tetramethylbutyl) phenol.

4-tert-OPnEO was part of ECHA's fifth recommendation of 6 February 2014 for the inclusion of substances in Annex XIV of REACh. Latest Application Date for its use is 4 July 2019; Sunset Date was set to 4 January 2021[1].

[1] COMMISSION REGULATION (EU) 2017/999 of 13 June 2017 amending Annex XIV to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

Under Use-1, SEBIA uses 4-tert-OPnEO for its detergent properties in the production of *in vitro* diagnosis (IVD) devices. 4-tert-OPnEO is used as the commercial product Triton™ X-100, supplied by Sigma-Aldrich, to manufacture one key product range in SEBIA's portfolio: the HYDRAGEL® range.

The main properties sought-after with 4-tert-OPnEO under Use-1 include:

- Being a non-ionic surfactant in order to not denature target proteins and to ensure interactions required for an efficient separation of sample components;
- Having an HLB (Hydrophilic-Lipophilic Balance) value comprised between 13 and 15 to ensure a good solubilisation of proteins of biological interest and thus their good distribution along the electrophoresis gel;
- Providing a similar level of analytical performances to that of Triton™ X-100, and more particularly, in terms of sensitivity;
- Providing a high level of clinical and diagnostical performances.

Under Use-1, 4-tert-OPnEO is used at two sites located in Lisses, in the Île-de-France region, France, and in one of SEBIA's subcontractors, REXOR, located in Paladru, in the Rhône-Alpes region.

2.2.1. SEBIA's site: Lisses

In 2004, SEBIA installed its manufacturing operations and headquarters to a newly developed site in Lisses, 27 Rue Léonard de Vinci, close to the Evry Bio-Park in France. This facility covers an area of over 52,650 m², and about 17,500 m² of buildings that include all business, R&D, production, storage and sales functions.

The installations of Lisses are "classified" and submitted to the technical requirements of the prefectoral order ("Arrêté prefectoral") of 28 October 2014[2], because of the presence of refrigerants in the site. More particularly, these requirements concern:

- prevention of water pollution and monitoring of COD, pH, temperature, specific pollutants (phenols, chromium VI, cyanides, AOX, arsenic and derivatives, total hydrocarbons and metals) quantities by samples collection;
- prevention of air pollution;
- waste management (recovery, recycling, secured waste storage, …);
- prevention of noise pollution and vibrations;
- prevention of risks and especially firefighting by ensuring the most suitable techniques and resources, and by identifying the parts of the installations which are at higher risk of disaster.

[2]http://www.essonne.gouv.fr/content/download/16531/145167/file/RAA+n%C2%B0+091+publi%C3%A9+le+6+novembre+2014+-+tome+1.pdf

Moreover, SEBIA has established an environmental management system and organisational policy, which is ISO 14001-2015 certified. SEBIA has also obtained the ISO 13485-2016 certification for research, development, production and sales of reagents and equipment for *in vitro* biological analysis. SEBIA is a FDA-accredited company and strictly follows the GMP (Good Manufacturing Process) standards.

Figure 2. Site of Lisses

2.2.2. REXOR's facilities: Paladru

REXOR, one SEBIA's subcontractor, is located 172 Rue Saint-Michel, 38850 Paladru. Created in 1954, REXOR is specialized in the plastic film processing, and employs 98 people to develop, manufacture and market a wide range of products using its four core businesses of plastics metallization, formulation, coating and cutting. The industrial park located on a total area of 40,160 m^2 including 10,650 m^2 of buildings, consists of three coating machines, one metallization machine and seven cutting machines. REXOR relies on Research and Development activities, allowing to expand and explore the resources of its h technologies. REXOR generates 18 million euros in turnover, 50% of which is exported to more than 30 countries.

Concerning environmental issues, the REXOR site is classified under authorisation for three activities:

- Storage and use of flammable solids (ICPE section n°1450);
- Heat transfer heating (ICPE section n°2915);
- Coating of plastic films (ICPE section n°2940).[3]

The site generates 100 tons of special industrial waste (DIS) and 300 tons of non-hazardous industrial waste (DIB) per year. The different types of waste are treated by approved companies with at least one energy recovery.

In addition, as part of a global environmental management policy, REXOR has been ISO 18001 and 14001 certified since May 2017. Thus, the design and manufacture of flexible support developed with the appropriate coating and cutting machines are subject to regular audits, attesting to their compliance with the requirements of the standard. In addition, the company also installed an incinerator in 2015 to eliminate any possible VOC emissions. In June 2017, it has been ISO 9001 certified too.

[3]http://www.isere.gouv.fr/content/download/30977/233679/file/DDPP-IC-2017-04-18%20REXOR%20APC_PALADRU.pdf

For SEBIA, REXOR manufactures the plastic support for gels (HYDRAGEL® range) by coating the plastic film by a solution containing 4-tert-OPnEO, directly supplied by SEBIA: 10 people are concerned by the use of Triton™ X-100 in the context of the production of plastic gel support.

Figure 3. REXOR's facilities

2.3. Elements of context

HYDRAGEL® assays that are concerned by Use-1 of the present application for Authorisation are used to isolate by gel electrophoresis specific proteins from and thus to diagnose various pathologies. In this context, samples of serum, urine, cerebrospinal fluid (CSF), plasma, … are analysed in view to detect blood diseases, haemoglobin abnormalities, cancers or infectious pathologies.

In what follows are detailed both the gel electrophoresis principle and the different pathologies diagnosed through HYDRAGEL® assays.

2.3.1. Agarose gel electrophoresis: principle and uses

2.3.1.1. General principle

Electrophoresis is a well-established separation technique used in a range of healthcare applications. The molecules to be separated are deposited on a support, each end of which is in contact with a buffer solution. In each buffer solution is an electrode. The electrodes are connected to a power generator creating an electric field which makes molecules migrate at different rates through the buffered medium. The migration speed is then different for the charged molecules, depending on specific characteristics of the molecules and electrophoresis conditions.

The amphoteric properties of proteins due to the presence of free carboxylic and free amino groups at the end of protein which can react with acids and bases, determine the charge of the proteins according to the pH. In acidic medium protein carries positive charges at amino group and in alkaline medium it carries negative charges at carboxylic group.

The molecules with larger charge-to-size ratios migrate at a faster rate than larger cations with smaller charges.

Consequently, in separating compounds into their constituent parts, important differences / abnormalities can be detected which ultimately lead to its use in diagnostic applications. (***Fig. 4***).

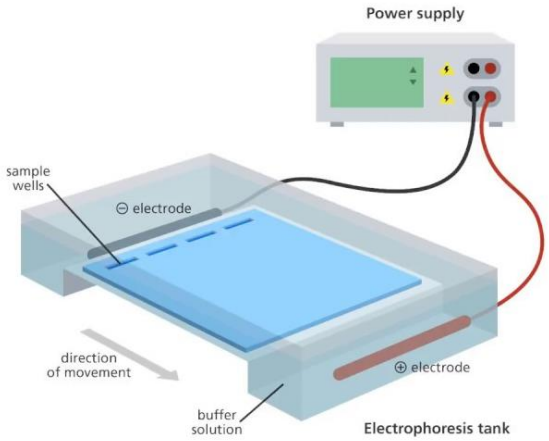


Figure 4. Gel electrophoresis scheme

2.3.1.1. Use of agarose: role in proteins separation

Agarose gel electrophoresis is a method used to separate a mixed population of macromolecules such as DNA or proteins extracted from blood, urine or cerebrospinal fluid samples in a matrix of agarose, one of the two main components of agar. The proteins may be separated by charge and/or size and the DNA and RNA fragments by length.[4] Biomolecules are separated by applying an electric field to move the charged molecules through an agarose matrix.

Indeed, agarose gel is a three-dimensional matrix formed of helical agarose molecules in supercoiled bundles that are aggregated into three-dimensional structures with channels and pores through which biomolecules can pass.[5] The 3-D structure is held together with hydrogen bonds and can therefore be disrupted by heating back to a liquid state. So, agarose gel has large pore size and good gel strength, making it suitable as an anti-convection medium for the electrophoresis of large molecules. Moreover, the agarose polymer contains charged groups, in particular pyruvate and sulphate.

Consequently, proteins, which are charged negatively, move towards the positively charged anode during electrophoresis. However, the migration of proteins in solution, in the absence of a gel matrix, is independent of molecular weight during electrophoresis.[6,7] The gel matrix is therefore responsible for the separation of

[4] Kryndushkin D. S., Alexandrov I. M., Ter-Avanesyan M. D., Kushnirov V. V. Yeast [PSI+] prion aggregates are formed by small Sup35 polymers fragmented by Hsp104. *J. Biol. Chem.* ***2003***, 278 (49), 49636–43.

[5] Sambrook J., Russell D. Chapter 5, protocol 1. Molecular Cloning - A Laboratory Manual. **1** (3rd ed.). p. 54.

[6] Zimm B. H., Levene S. D. Problems and prospects in the theory of gel electrophoresis of DNA . *Quarterly Rev. Biophys.* ***1992***, 25 (2), 171–204.

proteins by size during electrophoresis. In this context, as smaller molecules move through the agarose, the movement of larger structures such as globular proteins, lipoproteins or fatty acids is more likely to be impeded and slowed down by collisions with the gel matrix: the molecules of different sizes can therefore be separated in this sieving process.[4]

Finally, the separated molecules can be visualized by staining (ethidium bromide is frequently used for DNA and RNA) with a dye which intercalates into the major "grooves" of the molecule concerned. Regarding the staining used, it can fluoresce under UV light. The intercalation depending on the concentration of the molecule of interest, a band with high intensity will thus indicate a higher amount of molecule compared to a band of less intensity.[8] The different separated molecules can be then identified by comparison with known reference markers (***Fig. 5***)

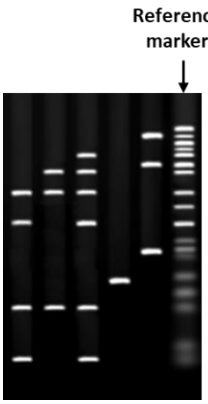


Figure 5. Bands in gels stained with Ethidium Bromide fluoresce under ultraviolet light

Regarding the nature of the molecule which has to be detected and quantified, different variants of the technique can be used.

Concerning the special cases of HYDRAGEL® applications under Use-1, these variants, namely immuno-electrophoresis, immunofixation and isoelectric focusing, are briefly described just below.

2.3.1.2. Immuno-electrophoresis

Immuno-electrophoresis, also called gammaglobulin electrophoresis, or immunoglobulin electrophoresis, is a method of determining the blood levels of three major immunoglobulins: immunoglobulin M (IgM), immunoglobulin G (IgG), and immunoglobulin A (IgA).

Immuno-electrophoresis is a technique that successively associates serum protein electrophoresis with immunodiffusion using immune sera. The proteins in

[7] Old R. W., Primrose S. B. . Principle of Gene Manipulation - An Introduction to Genetic Engineering (5th ed.). Blackwell Scientific. p. 9.

[8] Lee P. Y., Costumbrado J., Hsu C. Y., Kim Y. H., Agarose gel electrophoresis for the separation of DNA fragments. *Journal of Visualized Experiments*, ***2012***, 62.

the test serum are applied in the wells of the agarose gel and separated by electrophoresis. Antibodies are placed in the troughs and diffuse toward the separated proteins. A visible precipitin will form in a series of arcs in the agarose gel when an antigen-antibody reaction occurs. The shape and location of each arc are specific for known proteins. Unusual arcs are representative of abnormal or unknown protein. Although the density of the precipitation corresponds to the concentration of protein in each electrophoretic band, immuno-electrophoresis does not accurately quantify the amount of protein in the test serum.

Agarose buffered at basic pH (around 8.6) is traditionally preferred for the electrophoresis as well as the reaction with antibodies. The agarose was chosen as the gel matrix because it has large pores allowing free passage and separation of proteins but provides an anchor for the immuno-precipitates of protein and specific antibodies. The high pH was chosen because antibodies are practically immobile at high pH.

Thus, twenty precipitation arcs, corresponding to as many different proteins, are normally evidenced on serum immuno-electrophoresis (***Fig. 6***). The main isolated serum proteins are albumin, lipoproteins, orosomucoid, α-1-antitrypsin, complement, haptoglobin, α-2-macroglobulin, transferrin, C-reactive protein and especially immunoglobulins IgG, IgA and IgM.

These different markers thus make it possible to diagnose diseases such as multiple myeloma (IgG and IgA markers) and Waldenström disease (IgM markers).

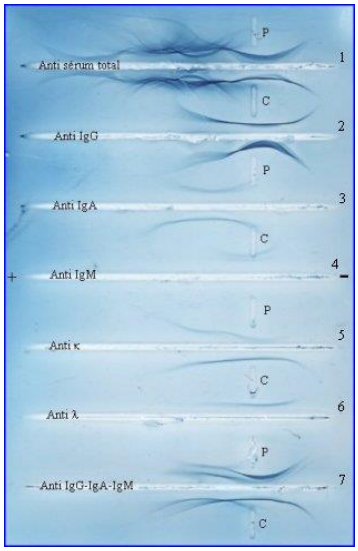


Figure 6. Immuno-electrophoresis result

According to the position and to the shape of the arcs, the different serum proteins can be characterized. The comparison of an arc obtained with the serum P with the corresponding arc obtained with the serum C (control) makes it possible to determine whether the protein involved is or is not at an abnormal concentration in the serum to be analysed. If the arc is closer to the channel, is thicker or more coloured, the serum protein characterized by the precipitating antibody is at an abnormally high concentration.

2.3.1.3. Immunofixation

Immunofixation is an immunological technique to identify and specify the typing of a monoclonal immunoglobulin (IgG, IgA or IgM) in the serum or urine of a patient. It has supplanted immuno-electrophoresis and is really useful for the diagnosis and assessment of certain blood disorders such as myeloma.

Immunofixation, like immuno-electrophoresis, is a method of precipitation detection, that is, when the soluble immunoglobulin is brought into contact with the corresponding antibody, a precipitation phenomenon occurs. With immunofixation

assay, monoclonal immunoglobulins can be identified in a mixture, according to their electrophoretic mobility. To enable this identification, antibodies specific for these immunoglobulins are used. It consists of depositing serum (or urine) on a gel. After application of an electric current which allows the separation of proteins according to their size, antibodies specific for each type of immunoglobulin are deposited on the gel. Thus, after staining, more or less narrow bands coloured by amidoblack or Acid Violet appear on the gel, at the level where the different immunoglobulins are located (***Fig. 7***).

Immunofixation, like immuno-electrophoresis, is therefore divided in two stages:

1. The first step is the same for both techniques. It consists of depositing the serum or the urine containing immunoglobulins, on a gel, then separating the sample proteins including immunoglobulins, according to their electrophoretic mobility by migrating them under the effect of an electric field. This migration depends on the mass and the charge of the antigen. Once the immunoglobulins are separated, we can proceed to the next step.
2. The second step consists, after migration, to deposit each specificity of anti-immunoglobulin on the specific migration track. The presence of a monoclonal immunoglobulin results, when an antigen-antibody reaction occurs, in the appearance of a band after staining of the precipitated complexes. For example, if it is an IgG lambda, a band will be observed, both on the two tracks where the anti-IgG and the anti-lambda were deposited.

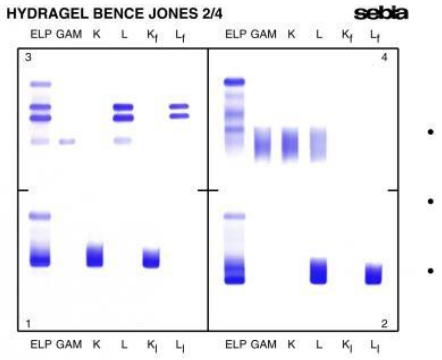


Figure 7. SEBIA's HYDRAGEL BENCE JONES immunofixation: separation and characterization of specific Monoclonal Free Light Chains or of any other monoclonal immunoglobulin

Thus, immunofixation has supplanted immuno-electrophoresis because it has the advantage of being:

- Faster (response time in less than three hours);
- More sensitive, so immunofixation may reveal an Ig passed unnoticed by immuno-electrophoresis, especially when it is present in small quantities (less than 1 gram/litre). In addition, the sensitivity can be increased by the use of enzyme (peroxidase) labelled anti-Ig antiserum. This is the case for the analysis of cerebrospinal fluid (CSF) which contains little proteins. An

increase in the amount of these proteins could be a sign of an inflammatory or immune disorder. These include multiple sclerosis (MS) and meningitis. Furthermore, the use of a high-resolution technique is particularly useful in this case;

- Partly automated and therefore achievable by a larger number of laboratories;
- Easier to read and interpret.

Here again, immunofixation makes it possible to diagnose numerous pathologies, according to the resolution adapted to the sample, and in particular those related to a monoclonal gammopathy. In addition to multiple myeloma and Waldenström disease, malignant lymphomas (chronic lymphocytic leukemia, non-Hodgkin lymphoma) and heavy chain disease are frequently detected by the immunofixation technique.[9] The immunofixation is considered as the gold standard.[10]

2.3.1.1. Isoelectric focusing

Isoelectric focusing (IEF), also known as electrofocusing, is a technique for separating different molecules by differences in their isoelectric point (pHi).[11,12] It is a type of zone electrophoresis, usually performed on proteins in a gel, that takes advantage of the fact that overall charge on the molecule of interest is a function of the pH of its surroundings.

IEF involves the addition of an ampholyte solution into an agarose gel matrix to create an immobilized pH gradient (IPG). This results in completely stable gradients except the most alkaline (>12) pH values. In this way, the immobilized pH gradient is obtained by the continuous change in the ratio of immobilines[13].

A protein that is in a pH region below its isoelectric point (pHi) will be positively charged and so will migrate towards the cathode (negatively charged electrode). As it migrates through a gradient of increasing pHi, however, the protein's overall charge will decrease until the protein reaches the pH region that corresponds to its pHi. At this point, as it has no net charge, its migration ceases (as there is no electrical attraction towards either electrode). As a result, the proteins become

[9] Heavy chain disease is a rare blood disease affecting lymphocytes. It is manifested by a pathological secretion of immunoglobulin heavy chains. There are therefore 3 types of disease, depending on whether the alpha, mu or gamma heavy chains are affected:
- Alpha chain disease causes chronic diarrhea and digestive malabsorption.
- The disease of the mu chains is characterized by a state close to chronic lymphoid leukemia.
- Gamma chain disease leads to an alteration of the general condition with fever, lymphadenopathy and hepatosplenomegaly.

[10] Aita M., Arantes L. C., Aita B. C.; Silva J. E. Comparison between immunofixation and electrophoresis for the early detection of relapsed multiple myeloma. *J Bras. Patol. Med. Lab*. ***2015***, 51 (6), 359-368.

[11] Bjellqvist B., Ek K., Righetti P. G., Gianazza E., Görg A., Westermeier R., Postel W. Isoelectric focusing in immobilized pH gradients: Principle, methodology and some applications. *J. Biochem. Biophysic. Meth.* ***1982***, 6 (4), 317–339.

[12] Righetti P. G. Isoelectric Focusing: Theory, Methodology and Application ***2000***, Elsevier.

[13] An immobiline is a weak acid or base defined by its pK value.

focused into sharp stationary bands with each protein positioned at a point in the pH gradient corresponding to its pHi. The technique is capable of extremely high resolution with proteins differing by a single charge being fractionated into separate bands.

Molecules to be focused are distributed over a medium that has a pH gradient (usually created by aliphatic ampholytes). An electric current is passed through the medium, creating a "positive" anode and "negative" cathode end. Negatively charged molecules migrate through the pH gradient in the medium toward the "positive" end while positively charged molecules move toward the "negative" end. As a particle moves towards the pole opposite of its charge it moves through the changing pH gradient until it reaches a point in which the pH of that molecules isoelectric point is reached. At this point the molecule no longer has a net electric charge (due to the protonation or deprotonation of the associated functional groups) and as such will not proceed any further within the gel. The gradient is established before adding the particles of interest by first subjecting a solution of small molecules such as polyampholytes with varying pHi values to electrophoresis (***Fig. 8***).

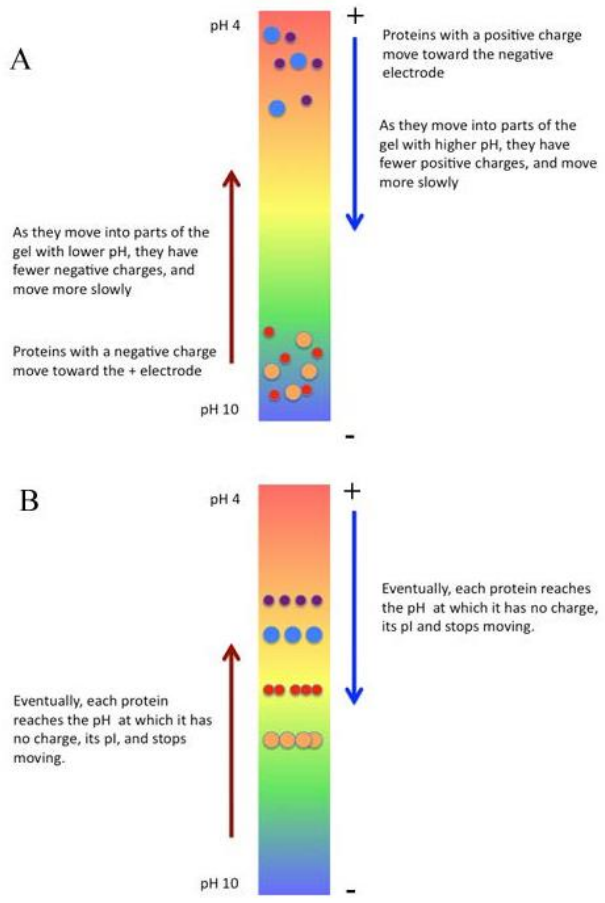


Figure 8. Isoelectric focusing: A. At the start of the run; B. at the end of the run

The method is applied particularly often in the study of proteins, which separate based on their relative content of acidic and basic residues, whose value is

represented by the pHi. Gels with large pores are usually used in this process to eliminate any "sieving" effects, or artefacts in the pHi caused by differing migration rates for proteins of differing sizes. Isoelectric focusing can resolve proteins that differ in pHi value by as little as 0.01.[14] Isoelectric focusing is the first step in two-dimensional gel electrophoresis, in which proteins are first separated by their pI and then further separated by molecular weight through SDS-PAGE.

2.3.2. Clinical use of HYDRAGEL® assays

In general, the HYDRAGEL® range allows the diagnosis of many diseases mainly associated with abnormalities of blood cells and gammopathies, but also with other pathologies related to enzymatic dysfunctions.

2.3.2.1. Blood proteins anomalies: Multiple Myeloma, Waldenström Disease and undetermined monoclonal gammopathies

Monoclonal diseases, also known as dysglobulinemia, refers to a qualitative or quantitative abnormality of immunoglobulins (blood proteins that play a role in the immune system by helping the body attack foreign elements such as virus and bacteria). Indeed, in case of monoclonal gammopathy, the system of regulation of the production of immunoglobulins dysfunctions, resulting in uncontrolled production and then in an auto-immune response causing the onset of certain diseases (***Fig. 9***).

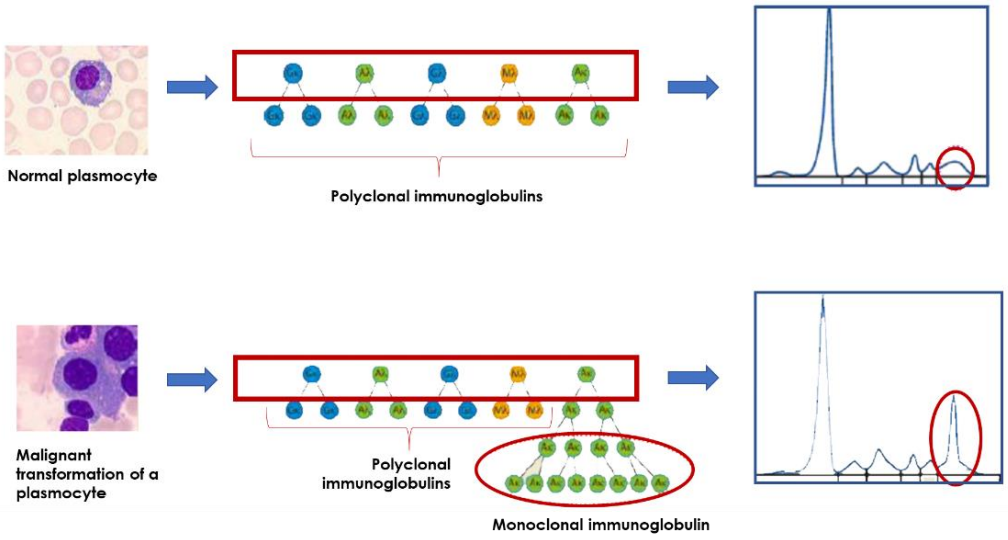


Figure 9. Origin of monoclonal immunoglobulins

ultiple Myeloma is a type of bone marrow cancer which results from the abnormal

[14] Stryer L. Spektrum Akademischer Verlag. *Biochemie* ***1996,*** 50.

secretion of one of these immunoglobulins. They are produced by plasmocytes which, in turn, are derived from white blood cells produced in the bone marrow. In Multiple Myeloma, white blood cells in the bone marrow become cancerous and reproduce uncontrollably, causing an overproduction of immunoglobulins (antibody proteins) which together form a tumour called plasmacytoma. A collection of these tumours ultimately crowds out the normal blood-forming cells and prevents them from functioning effectively. This leads to a breakdown in the body's ability to neutralise foreign elements (***Fig. 10***).

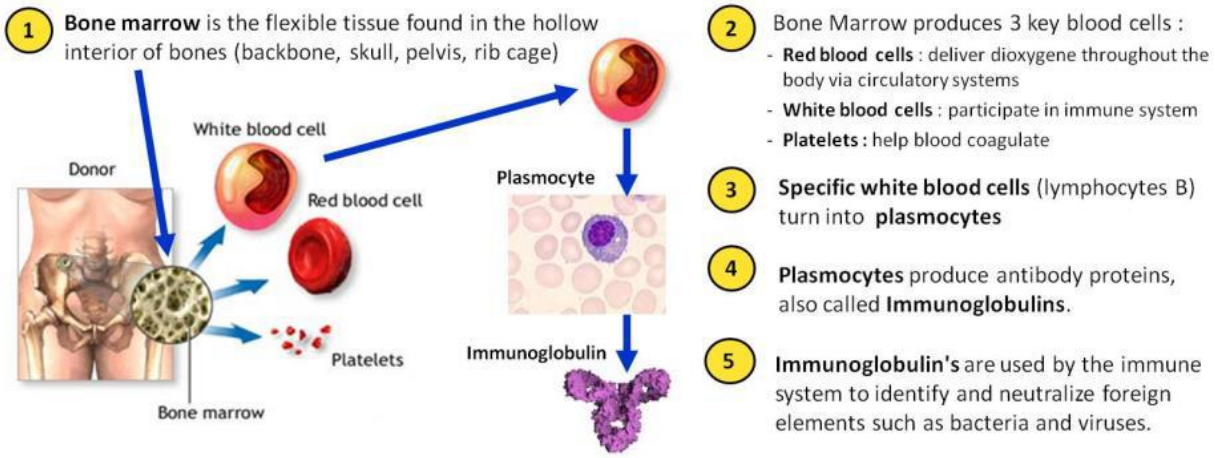


Figure 10. Multiple Myeloma mechanism

This disease, which affects about 7 out of 10,000 individuals per year, usually starts after age 50 and is twice as common in men as in women. It is reflected among other things by bone pain and spontaneous fractures. the most affected bones are rachis, ribs, skull, basin and humerus.

Electrophoresis testing is the referential standard for Multiple Myeloma screening and monitoring. A simple initial screening procedure using specific reagents (proteinogram) reveals abnormal immunoglobulin secretion, the key feature of Multiple Myeloma. If anomalies are discovered, as described previously, a test using immunofixation and immuno-typing[15] reagents can be achieved to qualify monoclonal peaks in order to determine the type of Multiple Myeloma afflicting the patient. Besides electrophoresis, no other test can be used to perform early stage diagnosis of Multiple Myeloma accurately, inexpensively and non-invasively.

[15] An alternative to immunofixation is immunotyping performed by capillary electrophoresis. This technology consists on an immuno-subtraction based on the disappearance of the monoclonal peak found by electrophoresis. This technique has the advantage of being automatic, but is less sensitive than immunofixation.

The **Waldenström disease** is another monoclonal gammopathy, characterized by the proliferation of lymphocytes B in the bone marrow, more rarely in the ganglia and spleen. These abnormal lymphocytes all produce the same antibody, called “monoclonal immunoglobulin M” (IgM). The electrophoresis of the proteins makes it possible to analyse the antibodies present. The presence of a large quantity of the IgM produced by the abnormal lymphocytes then results in a "monoclonal peak".

This disease is rather rare since there are only 3 to 5 cases per million inhabitants per year. It is possible to see some symptoms such as tingling in the legs, headaches, bleeding disseminated throughout the body (gums, mouth, nose) and a decline in vision or hearing.

Beyond these abnormalities today perfectly identified, there are nevertheless some cases of **monoclonal gammopathies of undetermined significance (MGUS).** These are observed mainly in the elderly and are more common in men than in women. This pathology is a "pre-cancer" and can evolve in 1% of cases per year to a myeloma. Such a diagnosis then involves a lifetime monitoring, semi-annual, based on electrophoresis of blood proteins, or even urinary proteins. It is the disease most diagnosed by SEBIA techniques (50% of cases).

2.3.2.2. Haemoglobin abnormalities: Haemoglobinopathies and thalassemia

Among the most known haemoglobinopathies, **sickle cell disease** is the most prevalent in the world. Resulting from a genetic mutation "false sense", this disease mainly affects the inhabitants of Africa and of the United States and the Caribbean. The disease is reported in the infant but the first signs do not appear before the age of 6 months. In the basal state, it is characterized by an anaemia which is reflected in a mucocutaneous pallor and then in cardiac signs. Electrophoresis is again the procedure of choice for confirmation of this kind of pathology.

Thalassemia are also concerned by this type of diagnosis technique. Indeed, resulting from a total or a partial deficit of haemoglobin chains, this anomaly can be easily identified by electrophoresis. There are two main families of thalassemia (***Fig. 9***):

- **β-thalassemia**, which are characterized by a wrong expression of haemoglobin, leading to anaemia or even a very severe form of sickle cell disease. The highest densities are described in Mediterranean countries (Italy, Sardinia, Sicily, Greece, ...);
- **α-thalassemia** whose frequency is even more important in Africa, Asia and around the Mediterranean. In general, they have clinical consequences only in the most severe forms, but in most cases, they are expressed only in the form of a minor anaemia.

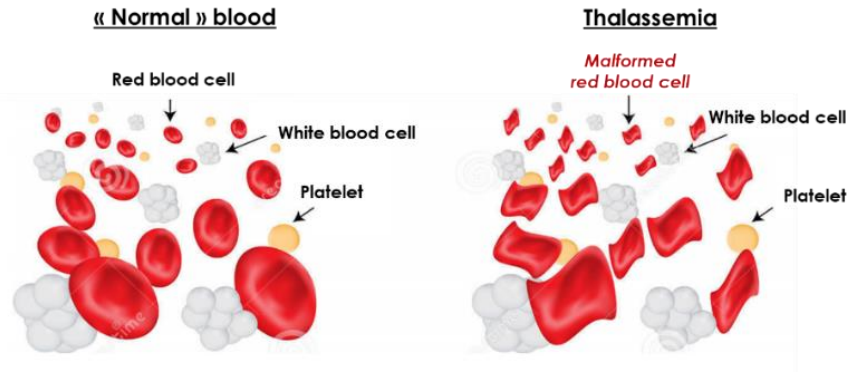


Figure 11. Normal blood and thalassemia profiles

2.3.2.3. Other pathologies covered by SEBIA's HYDRAGEL® assays

Other pathologies can be addressed by SEBIA, including malnutrition or multiple sclerosis. They are diagnosed by electrophoresis assays as they are characterized by:

- An **enzymatic dysfunction**, *via*:
  - An *increased enzymatic activity*: this is the case of ISO-PAL (PAL: alkaline phosphatase) generally symptomatic of a serious liver disease;
  - *Decreased enzymatic activity:* conversely, an ISO-PAL deficiency may indicate hypothyroidism, severe hepatic insufficiency, significant anaemia, scurvy or malnutrition;

- **Enzymatic overproduction**: the isoenzymes of CK (CK: creatine kinase), called CK-MB is thus specifically released in case of myocardial infarction; similarly, if LDH-1, one of the 5 isoenzymes of lactate dehydrogenase is present at a higher level than LDH-2, the diagnosis is also directed towards myocardial infarction.

- A **modified protein synthesis** through:
  - *Increased quantities*, as in the case of the presence of ß2 transferrin in the flow liquids (otorrhea, rhinorrhoea, etc. ...) that can reveal a break of the meningeal barrier;
  - *Lesser amounts*: for example, α-1 antitrypsin deficiency is a genetic disorder of variable clinical expression, initially described in patients with pulmonary emphysema (respiratory disease).

2.4. Products concerned

The use of 4-tert-OPnEO under Use-1 concerns a key family of products for SEBIA's portfolio: HYDRAGEL® product range.

2.4.1. HYDRAGEL® product ranges: corresponding equipment

HYDRAGEL® is a product range of gel electrophoresis essays for the identification and the quantification of proteins which are specific markers of certain pathologies. This range is manufactured for use on several automatons by professional laboratory personnel: HYDRASYS® and HYDRASYS® 2. "Focusing" versions, HYDRASYS® Focusing, and HYDRASYS® 2 Focusing, are especially dedicated to tests requiring a high-resolution performance, giving access to high specialization tests.

Figure 12. SEBIA's HYDRASYS®

The HYDRASYS® instruments allow simultaneous sample analyses on one gel. Sample application, migration, washing and staining are performed automatically.

The HYDRASYS® range comprises also "all-in-one" analysers for gel electrophoresis: indeed, the processed gels can be scanned by a densitometer, on-board the HYDRASYS® 2 SCAN and the HYDRASYS® 2 SCAN FOCUSING. The HYDRASYS® 2 SCAN carries out electrophoresis steps, from sample application to final reading. It is a fast and easy to operate instrument, offering a test menu of more than 60 HYDRAGEL® programs.

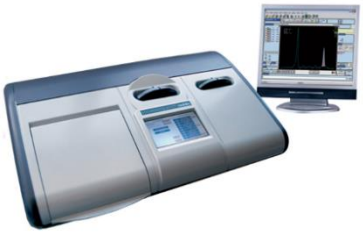


Figure 13. HYDRASYS® 2 SCAN

The pipetting equipment Assist and the standalone densitometry scanning system GELSCAN, used to quantify the protein fractions, complete this analytical range.

Figure 14. GELSCAN® scanning system

Figure 15. ASSIST® pipetting system

2.4.2. HYDRAGEL® product ranges: kits composition

HYDRAGEL® kits are generally composed of the following elements:

- **Agarose gels** ("ready-to-use"): on which is achieved the protein separation (the process of coating of the plastic support, carried out by REXOR for SEBIA is detailed in section 3.5.1);
- **Buffered strip** ("ready-to-use") acting as reservoirs for electrophoresis buffers and ensuring contact between the gel and the electrodes they are placed on the electrodes of the migration module;
- **Dye** ("stock solutions"), with (or not) its corresponding diluent, required in the staining of proteins and thus visualization of the electrophoregram; **Applicators** used to deposit samples on the electrophoresis gel;
- **Filter papers** to absorb the gel buffer or any solution in excess.

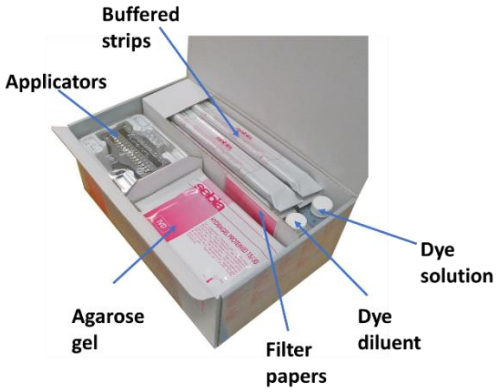


Figure 16. Classical composition of HYDRAGEL® kits

According to the kind of test, other reagents solutions can be added (like antisera containing, for example, immunoglobulins), mainly in immuno-electrophoresis or immunofixation assays.

2.4.3. HYDRAGEL® solution performances

According to the gel electrophoresis principle described previously, three types of protein separation occur according to:

- **The charge of the proteins, in a specific pH buffer**: indeed, the pH of the buffer solution induces the protein charge which is function of its isoelectric point of the particle (pH 3-12). The sign of the charge of the protein particle and the charge intensity is thus determined by the difference pH - pHi (***Table 2***);

If pH > pHi	Negative net charge (anion)	Migration to the anode
If pH<pHi	Positive net charge (cation)	Migration to the cathode
If pH = pHi	Nul net charge	No migration

Table 2. Protein charge according to the buffer pH and the protein pHi

- **The isoelectric point, in a specific pH gradient:** when the pH is applied as a gradient in the gel, the protein stops migrating when it reaches the level corresponding to its isoelectric point, at which the molecule is neutral;
- **The molecular weight of the proteins, in particular in a SDS-agarose gel**: the separation is carried out in denaturing conditions with Sodium Dodecyl Sulfate (SDS) which breaks protein di-sulfide bridges resulting in the loss of the ternary and the quaternary structures of the protein. Proteins are therefore separated according to their size and the "molecular sieve" effect. Finally, the molecular mass of separated proteins (stained with Coomassie blue, silver nitrate or fluorescent reagents) is determined in comparison with a mixture of proteins of known molecular masses, used as a standard.

Moreover, the maintenance of a constant and moderate electric field, the pH and the ionic strength (presence of ions necessary for the passage of the current) of the buffer, the nature and the composition of the support, the quality of the sample, but also temperature are essential parameters for optimal separation of the constituent proteins of the sample.[16]

Concerning the revelation and the reading, the concentration of the sample, sufficient to be detectable, and the choice of the dye are also to be taken into consideration, in particular for the dye, as part of the improvement of **resolution**, relative to the diffusion of the proteins in the gel (better resolution if proteins well separated and perfectly fixed in the gel) and of **sensitivity**: indeed, a test is considered as a sensitive assay when it makes it possible to visualize proteins present in very small quantities. The choice of the dye is therefore essential.

[16]https://www.sjbm.fr/images/cahiers/2003-Bioforma-28-Immunoglobulines%20monoclonales.pdf

Thus, in general, electrophoretic gel tests are selected on the basis of the following criteria[17]:

- **Reproducibility**: Essential to validate the results obtained, it depends on sample concentrations, quality of biological samples (presence of inhibitors, defects in their conservation) and the quality of pipetting. In fact, inaccuracy is usually determined by the coefficient of variation (CV)
- **Accuracy**: The accuracy (or appropriateness) of a method is the quality of the adequacy between the estimation of a measured value (X) and the true value (X_0), expressed through the coefficient of accuracy $CE = (X-X_0 / X_0) \times 100$[18].
- **Sensitivity**: The sensitivity is evaluated through the limit of detection (LoD) and the limit of quantification (LoQ)[19]. The sensitivity of a test is determined on a population of patients known to have the target disease because it has undergone a baseline test. It is defined by the proportion (%) of patients who have the desired disease and whose test is positive, in other words by the proportion of patients with target disease that the test detects correctly (true positives). In contrast, the proportion of patients with disease that the test did not identify are false negative results. The sensitivity is given by TP/(TP+FN) and is always evaluated in relation to a patient population by the targeted pathology (***Table 3***). This evaluation can also be qualitative and is based on both:
    - The highest dilution at which it is possible to identify the desired band at the highest dilution
    - The lowest concentration of detectable targeted protein
- **Specificity** which is coupled to sensitivity and which should allow to establish the probability that the test is negative in the case of samples of patients not affected by the disease. Quantitatively, this is calculated according to the expression TN/(TN+FP) (***Table 3***).

	ILLNESS	NO ILLNESS
Positive test	TP (true positive)	FP (false positive)
Negative test	FN (false negative)	TN (true negative)

Table 3. Test results vs illness for sensitivity/specificity evaluation

[17] ANSES Guide de validation des techniques d'analyse, ***2015***, https://www.anses.fr/fr/system/files/ANSES_GuideValidation.pdf

[18] Nab M. Etude comparative de l'électrophorèse sur gel et l'électrophorèse sur l'automate CAPILLARYS®, Ecole supérieur des sciences et techniques de la santé de Monastir Tunisie - Diplôme de technicien supérieur en biologie médicale ***2010***, Chap. 4.2.

[19] The Quantization Limit is defined as the smallest concentration that can be quantified with an acceptable intra-laboratory precision level set at 20% of coefficient of variation.

These two criteria are often completed by two other evaluation data:

- The **positive predictive value** (PPV) which is the probability that the disease is present when the test is positive.
- The **negative predictive value** (NPV) which is the probability that the disease will not be present when the test is negative.

The required performance levels are therefore high and vary according to the experience to be achieved. It is thus admitted that very sensitive tests are especially useful to ensure that a disease is not present (few false negatives) while those that are very specific are useful to ensure that a disease is well present (few false positives).[20]

Consequently, gel electrophoresis is an efficient tool to separate various components from a sample, in view of identifying proteins and thus diagnosing a large panel of pathologies. In this purpose, HYDRAGEL® solutions offer:

- An efficient **quantification**, which may be useful for clinical interpretation;
- **Less false positives** because the process does not require an excessive handling of the samples: the sample application, the electrophoretic migration, the drying, the staining, the destaining and the final drying are carried out according to a totally automated sequence;
- A **high resolution**: an optimal focalization of the fractions is obtained by applying a very thin sample application using the SEBIA proprietary applicators;
- A **good sensitivity**: internal sensitivity studies have demonstrated that proteins can be detected in a sample at a very low concentration;
- A **specificity** guaranteeing that the observed signals corresponds to the target proteins;
- A **full traceability** is obtained, by connecting the HYDRASYS®® to the ASSIST® pipetting station.

In this way, many publications report to date the performance of HYDRAGEL® tests, both in terms of reproducibility, sensitivity or specificity. Positive and negative predictive values confirm the relevance of such analytic methods too. Some of these results obtained in the electrophoresis of CSF and/or serum proteins (γ-globulins, β1- and β2-globulin, α1- and α2-globulin, albumin) are as follows:

[20] Bustin *et al.* The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments*. Clin.l Chem. **2009***, 55 (4), 611–622.

Publication	SEBIA HYDRAGEL® device	Reproducibility[21] (CV%)	Sensitivity (%)	Specificity (%)	PPV	NPV
1[22]	HYDRAGEL® PROTEIN 15/30	<5%	93.5	98.5	ND	ND
2[23]	HYDRAGEL® protein 15/30	ND	90.9	81.0	89.4	84.1
3[24]	HYDRAGEL® IF	ND	44.0	85.0	85.0	80.0

Table 4. Results of various studies carried out on HYDRAGEL® assays

So, HYDRAGEL® solutions demonstrates excellent reproducibility, specificity and sensitivity in various studies. As described previously, many parameters have an influence on HYDRAGEL® performance levels. In this way, 4-tert-OPnEO plays a crucial role in the improvement of performance characteristics. In the special context of Use-1, sensitivity is highly increased thanks to wettability properties of Triton™ X-100. According to customers' requirements and in particular to sensitivity criteria, SEBIA HYDRAGEL® electrophoresis-based kits are considered by laboratories as reference routine tools in the diagnosis of pathologies linked to blood abnormalities.

Finally, products concerned by Use-1 include (***Table 5***):

- **Dyes** (*2 references*) and **colouring bases** (*11 references*) allowing a good reading of separated proteins;
- **"Strip" buffers** (*12 references*), ensuring contact and thus migration between the gel and electrodes;
- **Fixing solutions** (*1 reference*) immobilizing the proteins in the gel at the expected migration level and as described in the corresponding kit leaflet;
- **Antiserum diluents** (*5 references*), optimizing the spreading of antiserum in the gel medium**.** Antiserum solutions contain immunoglobulins directed against human Ig G, conjugated to an enzyme. In the context of the immunofixation process of interest, the sample Ig G binds antiserum Ig;
- **Substrate solvents** (*2 references*), ensuring a better distribution of the substrate on the gel and thus a better separation of proteins;
- **Coated plastic support** (*1 reference*) produced by REXOR for SEBIA.

[21] The reference value is fixed at 2-3 % for albumin, and 4 à 5 % for γ-globulins.

[22] Lissoir B. *et al.* Electrophorèse des protéines sériques, Ann Biol Clin ***2003***, 61 : 557-62 - http://www.jle.com/download/abc-260408-electrophorese_des_proteines_seriques_comparaison_de_la_technique_en_capillaire_de_zone_CAPILLARYS®_SEBIA--WyfA3X8AAQEAAEGSzlYAAAAB-a.pdf

[23] McCudden C. *and coll.* Performance Comparison of Capillary and Agarose Gel Electrophoresis for the Identification and Characterization of Monoclonal Immunoglobulins. *Am. J. Clin. Pathol.* ***2008***, 129, 451-458.

[24] Tagajdid M.R. and coll. Contribution of CSF/serum immunofixation to the diagnosis of inflammatory diseases of the central nervous system. *Revue Neurologique* ***2011***, 167 (3), 225-230.

HYDRAGEL® RANGES	NATURE OF THE SOLUTION	NUMBER OF SOLUTIONS REFERENCES INVOLVED	NUMBER OF KITS REFERENCES IMPACTED
HYDRAGEL® PROTEIN(E)	Dye	1 (ref. 56046)	7
	Strip buffer	1 (ref. 56187)	
	Coated support	1 (ref. 56302)	
HYDRAGEL® HR	Dye	2 (ref. 56106 & 56046)	7
	Strip buffer	1 (ref. 56196)	
	Anti-serum diluent	1 (ref. 56222)	
	Coated support	1 (ref.56302)	
HYDRAGEL® PROTEINURIE	Dye	1 (ref. 56106)	2
	Coated support	1 (ref. 56302)	
HYDRAGEL® β1-β2	Dye	1 (ref. 56046)	4
	Strip buffer	1 (ref. 56218)	
	Coated support	1 (ref.56302)	
HYDRAGEL® LIPOPROTEIN	Strip buffer	1 (ref. 56184)	3
	Coated support	1 (ref. 56302)	
HYDRAGEL® LIPOPROTEIN (E)+LP(A)	Strip buffer	1 (ref. 56186)	4
	Coated support	1 (ref. 56302)	
HYDRAGEL® LDL/HDL CHOL. DIRECT	Strip buffer	1 (ref. 56028)	4
	Substrate solvent	1 (ref. 56056)	
	Coated support	1 (ref.56302)	
HYDRAGEL® IF PENTA	Dye	1 (ref. 56046)	8
	Colouring base	1 (ref. 56328)	
	Fixing solution	1 (ref. 56600)	
	Strip buffer	1 (ref. 56191)	
	Coated support	1 (ref. 56302)	
HYDRAGEL® IF	Dye	2 (ref. 56046 & 56106)	18
	Colouring base	5 (ref. 56132, 56133, 56134, 56136, 56137)	
	Fixing solution	1 (ref. 56600)	
	Strip buffer	1 (ref. 56191)	
	Coated support	1 (ref. 56302)	
HYDRAGEL® IF/BENCE JONES	Dye	1 (ref. 56106)	2
	Colouring base	5 (ref. 56135, 56136, 56137, 56138, 56139)	
	Fixing solution	1 (ref. 56600)	
	Strip buffer	1 (ref. 56232)	
	Coated support	1 (ref. 56302)	
HYDRAGEL® BENCE JONES	Dye	1 (ref. 56106)	9
	Colouring base	5 (ref. 56135, 56136, 56137, 56138, 56139)	
	Strip buffer	1 (ref. 56191)	
	Coated support	1 (ref. 56302)	

HYDRAGEL® OUCHTERLONY	**Coated support**	1 (ref. 56302)	1
HYDRAGEL® HEMOGLOBIN(E)	**Dye**	1 (ref. 56046)	3
	Strip buffer	1 (ref. 56194)	
	Coated support	1 (ref.56302)	
HYDRAGEL® ACID(E) HEMOGLOBIN(E)	**Dye**	1 (ref. 56046)	3
	Strip buffer	1 (ref. 56290)	
	Coated support	1 (ref.56302)	
HYDRAGEL® APO E IF	**Anti-serum diluent**	1 (ref. 56222)	2
	Coated support	1 (ref.56302)	
HYDRAGEL® IEP	**Dye**	2 (ref. 56046 & 56106)	3
	Coated support	1 (ref. 56302)	
HYDRAGEL® CSF	**Strip buffer**	1 (ref. 56232)	4
	Anti-serum diluent	1 (ref. 56239)	
	Coated support	1 (ref. 56302)	
HYDRAGEL® CSF ISOFOCUSING	**Anti-serum diluent**	1 (ref. 56222)	2
	Coated support	1 (ref. 56302)	
HYDRAGEL® ISO-LDH	**Strip buffer**	1 (ref. 56240)	4
	Coated support	1 (ref. 56302)	
HYDRAGEL® ISO-PAL	**Substrate solvent**	1 (ref. 56040)	3
	Strip buffer	1 (ref. 56271)	
	Coated support	1 (ref. 56302)	
HYDRAGEL® ISO-CK	**Strip buffer**	1 (ref. 56240)	3
	Coated support	1 (ref. 56302)	
HYDRAGEL® A1AT ISOFOCUSING	**Antiserum diluent**	1 (ref. 56380)	2
	Coated support	1 (ref. 56302)	
HYDRAGEL® URINE PROFILE	**Dye**	1 (ref. 56106)	6
	Colouring base	2 (ref. 56140 & 54141)	
	Strip buffer	1 (ref. 56191)	
	Coated support	1 (ref. 56302)	
HYDRAGEL® β2 TRANSFERRINE	**Strip buffer**	1 (ref. 56194)	1
	Anti-serum diluent	1 (ref. 56239)	
	Coated support	1 (ref. 56302)	
HYDRAGEL® VON WILLEBRAND	**Anti-serum diluent**	1 (ref. 56327)	1
OTHER PRODUCTS SOLD SEPARATELY	**Colouring base**	10 (ref. 56132, 56133, 56134, 56135, 56136, 56137, 56138, 56139, 56142, 56328)	29 separate solutions & 4 solutions boxes
	Fixing solution	1 (ref. 56600)	
	Dye	2 (ref. 56046 & 56106)	

Table 5. SEBIA's products concerned by Use-1 (• *REXOR product*)

So, finally, each HYDRAGEL® range contain at least one solution including 4-tert-OPnEO representing thus 133 HYDRAGEL ® kits. Moreover,

- Excluding the HYDRAGEL® Von Willebrand kit, **the REXOR support is present in all the gel kits** (i.e. 132/133 references);
- Other **29 solutions and 4 boxes grouping several buffers containing 4-tert-OPnEO** and sold separately are concerned too.

HYDRAGEL® assays allow to detect and to quantify proteins involved in various pathologies. Identified as reference tools for proteins gel electrophoresis, these tests are largely used by laboratories and hospitals, and thus constitute the backbone of SEBIA's portfolio.

To date, with all the HYDRAGEL® range kits concerned by this Authorisation application, the ban on using 4-tert-OPnEO could jeopardise the survival of the company.

2.5. Supply chain

The supply chain of SEBIA's IVD devices can be described as follows:

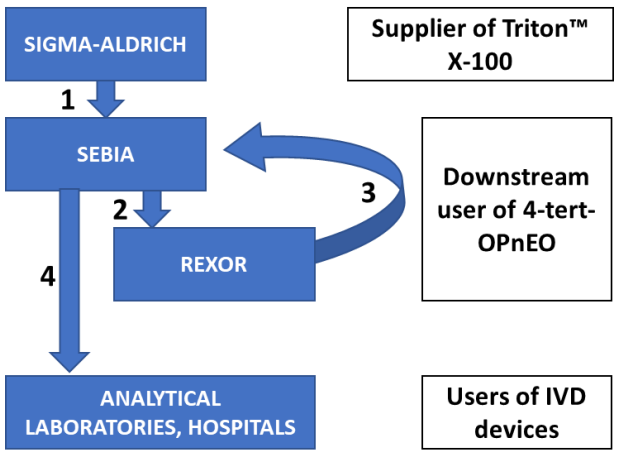


Figure 17 . Supply chain of SEBIA's IVD devices

To date, SEBIA supplies Triton™ X-100 based solution to REXOR which employs the corresponding solution to coat the plastic film employed as the electrophoresis support then included in IVD kits, as finished products.

As per Art. 56(3) of REACh, and according to the definition of SR&D activities provided in ECHA's guidance[25], end-user analytical activities (use of kits) performed by laboratories and hospitals are exempted from Authorisation.

Nevertheless, through this AfA, SEBIA wishes to cover the entire life cycle of the substance, including thus downstream users' activities linked to the use of SEBIA' kits containing the substance. **In this purpose, quantities of substance contained in the kits and supplied to end-users and their conditions of use are described in the Chemical Safety Report, while substance function in the final kit is specified in the section 4.1. These elements thus tend to justify the application of the SR&D exemption through the demonstration of the implementation of controlled operating conditions, as well as the importance of using 4-tert-OPnEO for the efficiency and the good realization of the IVD test.**

Moreover, in order to ensure the application of the controlled conditions for the use of the kits concerned by this AfA, SEBIA is committed, within the framework of this Application, to raising awareness of all its customers. In this purpose, SEBIA will use all the media at its disposal: training of user staff, communication via the Company's website, writing of a specific insert in the MSDS of the various kits and writing of a brochure especially dedicated to this issue.

Examples of MSDS including this special notice about the presence of the substance in the kit, a proposal of website content and a first version of the information brochure are reported, respectively, in Appendixes 2, 3 and 4.

2.6. Elements of context

2.6.1. General production process

The general production process of IVD reagents concerned by the application for Authorisation is synthetized as follows, regarding the type of solutions (***Fig. 17***).

In addition to production operations presented in these figures, several industrial steps are required for controlling and validating the quality of the products. A large volume of facilities and human resources is therefore conditioned to the production of reagents for IVD kits of Use-1.

[25] ECHA, Guidance on Scientific Research and Development (SR&D) and Product and Process Orientated Research and Development (PPORD), Version 2.0, November 2014.

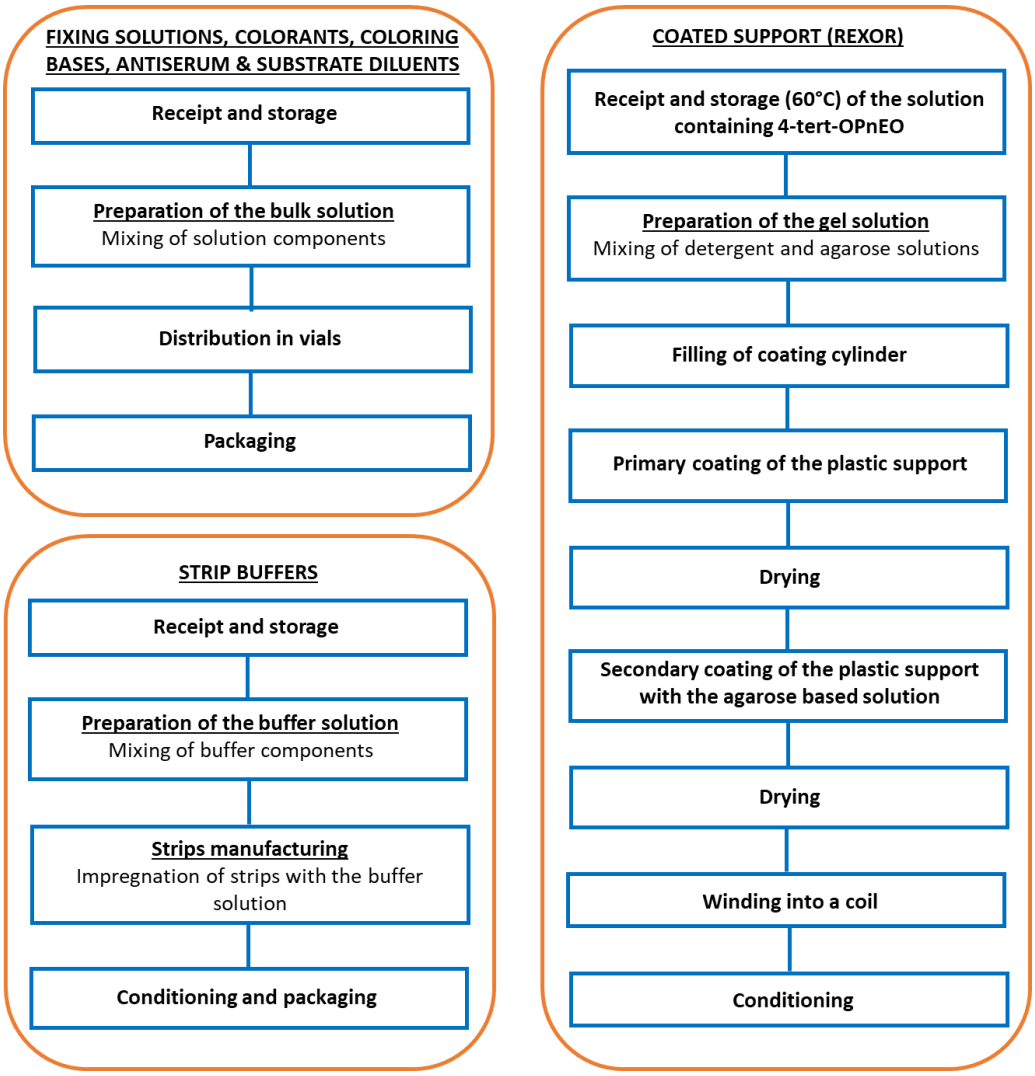


Figure 18. General production processes of reagents and additives solutions composing SEBIA kits and coated support supplied by REXOR

2.6.2. Market

On the basis of product, the reagent segment accounts for the largest share of the global electrophoresis market. This is primarily attributed to the increasing demand for protein separation electrophoresis for various applications such as biomarker discovery and protein mapping. Over the years, genomic and proteomic technologies have gained significant importance in fields of clinical diagnosis and drug discovery and development. These are some of the key factors driving the growth of the electrophoresis market, and in turn supporting the growth of the electrophoresis reagents market during the forecast period.[26]

[26] Markets and markets. Report Electrophoresis Market by Product (Gel Electrophoresis; Capillary Electrophoresis; Reagents; Imaging; Application; End User - Global Forecast to 2022, ***2016.***

Many ranges of SEBIA's products are based on protein separation by electrophoresis and are dedicated to the diagnosis of pathologies such as myeloma, for which SEBIA has 75% global market share, haemoglobin pathologies, other markers of metabolic disorders and, more recently, diabetes, where the Company has gained more than 40% of market share in France in three years. Nevertheless, in parallel, several competitors offer gel electrophoresis kits solutions dedicated to the identification of blood abnormalities too. The major methods and reagents for serum proteins separation are listed in the following table. According to a survey[27] carried on 948 laboratories, agarose gel remains the most used medium for gel electrophoresis: cellulose acetate represents only 1% of the gel uses. The same survey shows that SEBIA's HYDRASYS® technologies are largely used by laboratories: when they are associated to Amidoschwarz dye, the SEBIA's HYDRASYS® solutions are the major equipment used in France now, mainly with immunofixation coupling.

KIT NAME	MANUFACTURER	COLORANT USED	Albumin (CV%)	α1-globulin (CV%)	Γ-globulin (CV%)
HYDRAGEL Protein(e) HR	SEBIA	Amidoschwarz	0.3-1.4[28]	2.1-9.8[32]	1.3-5.6[32]
BIOMIDI Protéines	BIOMIDI	Amidoschwarz	4.4	11.1	6.0
SAS-1 Serum Protein SB	ELITechGroup (Helena Lab. Corp.)	Acid blue	2.0	11.4	9.4
Titan Protéines 2000	ELITechGroup (Helena Lab. Corp.)	Acid blue	3.3	17.0	3.6
SAS-MX serum Protein	ELITechGroup (Helena Lab. Corp.)	Acid blue	5.5	9.5	4.9
Titan III Protéines	ELITechGroup (Helena Lab. Corp.)	Red culvert	4.1	23.9	8.9

Table 6. Selected IVD agarose gel electrophoresis kits for protein electrophoresis: major solutions used in laboratories [29]

So, to date, Helena Lab. Corp. is the main SEBIA's competitor, in particular for kits dedicated to the diagnosis of Myeloma and the identification of Ig abnormalities-based pathologies. Haemoglobin anomalies are detected by several tests developed by Bio-RAD, Trinity, Tosoh and Arkray. Roche, Siemens and Abbott have also market shares in the HbA1c tests.

Nevertheless, these solutions cannot be considered as direct substitutes to HYDRAGEL® solutions as:

[27] ANSM (Agence Nationale de la sécurité du médicament et des produits de santé - National Agency of Drug Safety) - *Annales du contrôle national de qualité des analyses de biologie médicale*, ***2013***.

[28] Average values obtained for inter-assay reproducibility tests - Please refer to package insert of HYDRAGEL Protein(e) HR assay.

[29] Roger *et al.* Widely Used Types and Clinical Applications of D-Dimer Assay. *Laboratory Medicine* 47:2; 90-102.

- They use different dyes which have to be adapted to proteins to be determined and requiring different sensitivity levels depending on their potential and usual concentration in the sample;
- Even if albumin CV results remain low for all these techniques, the results dispersion obtained for the other globulins are generally better with HYDRASYS® than with other technologies;
- They require dedicated detection equipment and cannot be directly used on SEBIA's automated equipment;
- In addition, today, HYDRASYS® solution is considered as a reference tool for many laboratories: according to numerous publications and reports, SEBIA's gel products are much more frequently employed by kits users than any other solution. The relative contexts of such a use are numerous: pathologies diagnosis and follow-up, comparative studies with other technologies, ... It would be unrealistic to want to list all the publications concerning the HYDRAGEL® range and its performances, but as an illustration, Smith *et al.*[30] studied performance properties of the HYDRAGEL® 15 HR system in comparison with those of immunofixation methods. Sensitivity results were therefore much higher, testifying to the efficiency of the SEBIA solution.
- So, given SEBIA's market share for these assays, it is very unlikely that one actor will be able to upscale its production so as to cover SEBIA's uses.

These elements are further detailed in section 3 of the present document.

2.7. General methodology

Endocrine disrupting properties of 4-tert-OPnEO degradation product make it difficult to derive a dose-response relationship that would quantitatively link substance release and environmental impacts.

In these conditions, ECHA identified three elements required in order to conclude that the benefits of continued use outweigh the risk[31]. These elements, along with a comment regarding where they have been developed in the present document as follows:

[30] Smith J., Raines G., Schneider H. G. A comparison between high resolution serum protein electrophoresis and screening immunofixation for the detection of monoclonal gammopathies in serum. *Clin. Chem. Lab. Med.* ***2018***, 56 (2), 256-263.

[31] ECHA, SEA-related considerations in applications for authorisation for endocrine disrupting substances for the environment, specifically OPnEO and NPnEO (SEAC/37/2017/03), Helsinki, ***30 November 2017***.

ELEMENT	COMMENT
Monetised estimate of the benefits of continued use	Monetised costs as well as qualitative impacts of the "non-use" scenario are detailed in section 5.
Quantified release estimates accompanied with a qualitative description of where the releases occur	Release estimates are detailed in the Chemical Safety Report; a detailed description of the potentially receiving medium is provided in section 3.4.1
Qualitative description of the potential impacts	A qualitative description of the potential impacts is detailed in section 3.4.2

Table 7. Elements of demonstration that the benefits of continued use outweigh the risk

In addition to these elements and by analogy with ECHA's approach regarding the evaluation of restriction reports and applications for authorisation for PBT and vPvB substances[32], a cost-effectiveness analysis will be provided.

Cost-effectiveness analysis (CEA) as a decision supporting methodology aims at determining the most (economically) efficient way to achieve a regulatory objective. In the context of an application for Authorisation under REACh, and since there are great difficulties to characterise the relationship between volume of effluents and environmental impacts, it will be assessed:

- **Costs per reduced unit emission**, i.e $\frac{Costs\ (€)}{kg\ emission\ avoided}$
- **Costs per reduced unit consumption**, i.e $\frac{Costs\ (€)}{kg\ consumption\ avoided}$

Costs per reduced unit emission will be used as a primary cost-effectiveness characterisation ratio; costs per unit consumption will also be calculated as a sensitivity analysis in view of provided a broader perspective regarding the overall potential impacts of the application.

2.7.1. Scope of the AfA

Key elements of the scope of the AfA are provided in ***Table 8*** below:

[32] ECHA, Evaluation of restriction reports and applications for authorisation for PBT and vPvB substances in SEAC, ***2016***.

SCOPE	COMMENT
Temporal boundary	Twelve years post-sunset date: 2021 – 2033. See table below for a description of the triggering period for each impact.
Geographic boundaries	Direct impacts concern France. Indirect impacts for SEBIA's supply chain customers cover a worldwide scope.
Economic boundaries	Direct economic impacts for SEBIA (loss of profits) Indirect impacts for SEBIA's value chain (end-users)

Table 8. Scope of the AfA

Impact periods for these two scenarios are as follows:

CATEGORY	IMPACT	IMPACT PERIOD
Economic impacts	**Direct impacts: loss of profits and revenues**	12 years
	Indirect impacts: loss of markets	Variable: 3 to 12 years depending on the frequency of tenders
Social impacts	**Impacts on employment**	1 year
	Medical impacts	Several months to years depending on medical and market situations
Wider economic impacts	**Negative impacts related to market disruption**	Several months to years depending on market situations
	Opportunities for competitors	Several months to years depending on market situations

Table 9. Impact periods for the two product ranges of Use-1

Present value is set in 2019, at the date of submission.

2.7.2. Actualisation

All final monetised results of this document are expressed in present value (PV).

2.7.2.1. Discounting

Comparing costs and benefits during different periods of time to present values requires the use of discounting technique to translate future costs and benefits into present-days values to account for the time value of money

The choice of discount rate is important since it can affect the cost-benefit results of the analysis. The higher the discount rate, the lower the future benefits and costs values will be, as compared to present values.

In our methodology, we deliberately chose to use two different discount rates depending on the type of future impacts evaluated.

Thus, future human health costs described in the "applied for use" scenario of this dossier will be evaluated using a lower discount rate that the one used to consider economic impacts in the "non-use" scenario. This difference is related to the different "nature" of these impacts and aims to reflect the society's rate of time preference with respect to health risks.

As per ECHA's guidelines, the calculation of discounted values is performed on an annualised basis, with the following formula:

$$PV = \sum_{n=1}^{n=t} F_n(1+r)^{-n} = \frac{F_1}{(1+r)} + \frac{F_2}{(1+r)^2} + \cdots + \frac{F_t}{(1+r)^t}$$

Considering:

- PV = present value
- F_n = future costs at year n
- r = annual discount rate
- t = last annuity of the discount period

Based on ECHA's recommendation[33], a 4% discounting rate is used to assess the future cost/benefits values for impacts not related to health matters.

2.8. Substitution strategy

A significant work of research for the substitution of Triton™ X-100 in the production process of HYDRAGEL® assays led to identify several potential alternatives:

These alternatives, however, have yet to be further investigated, implemented and qualified and will therefore not be available before the sunset date of 4-tert-OPnEO.

2.9. Presentation of the "applied for use" and "non-use" scenarios

2.9.1. "Applied for use" scenario

Under the "applied for use" scenario, SEBIA will pursue the use of 4-tert-OPnEO in the production of products concerned by Use-1 during the period of time necessary to develop, implement and validate an alternative process, thereby securing both its activity and the supply of IVD tests to laboratories and hospitals.

[33] ECHA, Guidance on the preparation of socio-economic analysis as part of an application for Authorisation, ***2011***.

Environmental risks and impacts of the “applied for use” scenario are respectively detailed in section 3.4.2.

2.9.2. “Non-use” scenario

The most likely “non-use” scenario for Use-1 is following: with the ban on the use of 4-tert-OPnEO, SEBIA will have to cease the production of HYDRAGEL® assays.

Among the arguments that make it impossible to consider an alternative “non-use” scenario (performance degradation, relocation or sub-contracting outside the European Union) are the intrinsic characteristics of the production process of HYDRAGEL® that:

- requires an extremely high level of know-how and a major human factor;
- follows a complex internal and external qualification process;
- concern the entire gel electrophoresis tests range with numerous detergent-based reagent solutions incorporated in the kits;
- requires an extremely low level of batch-to-batch variability according to end-user’s performance specifications.

A comprehensive description of these elements is provided in the following section.

3. "APPLIED FOR USE" SCENARIO

Under Use-1, SEBIA uses 4-tert-OPnEO as a detergent in the manufacture of reagents for *in vitro* diagnostic assays based on electrophoretic techniques.

The main functional properties sough-after by SEBIA with 4-tert-OPnEO include non-ionic surfactant properties and defined HLB (Hydrophilic-Lipophilic Balance) value in order to well solubilize proteins of interest, and good wetting properties in view of ensuring an efficient distribution of samples, reagents and other additives in the gel and other electrophoresis components. These properties are mandatory to obtain similar levels of analytical and clinical performances to that obtained with Triton™ X-100, especially a high sensitivity.

3.1. Analysis of substance function

Triton™ X-100 is a non-ionic surfactant with detergent properties. Its substitution in the context of the production of HYDRAGEL® assays has to take into consideration: possess physico-chemical properties that allow for the same or better analytical and clinical performances compared to that of the Triton™ X-100 as well as present a lower level of risk for human health and the environment.

3.1.1. Functional properties

4-tert-OPnEO is used in the context of Use-1 in view of ensuring the dissolution and the solubilisation of substrates contained into the samples and other reagents required in the achievement of the test, and allowing their good spreading in the electrophoretic apparatus.

So, as to play its role within the production process of HYDRAGEL® assays, potential alternatives need to possess several properties:

1. **Optimization of the molecules migration by solubilization and stabilisation of proteins and improvement of test sensitivity**

In order to obtain expected results, certain physico-chemical conditions are necessary for the proper performance of the test and in particular the good diffusion of the proteins in the gel. Thus, as the migration depends mainly on the charge and the mass of the protein, it must be ensured that no other parameter can intervene in the migration and modify the result. That's the reason why the perfect solubilization of the proteins within the gel is essential. This takes place firstly during the deposition of the different samples in the electrophoresis wells which has to be perfectly homogeneous and secondly throughout the test.

Once solubilized, the proteins (and other molecules such as colouring agents, fixatives, etc.) can thus diffuse continuously. This continuity is both physical, the different elements composing the electrophoresis system being connected to each other (in particular through the buffer solutions which ensure the contact between electrodes and electrophoresis gel) and chemically, the composition of the medium being identical at any point of the system.

Triton™ X-100 (***Fig. 19***) is a non-ionic surfactant, and therefore have with limited sensitivity to both pH and the ionic strength of the environment. This characteristic is necessary to eliminate issues related to the denaturation of proteins in the absence of electrostatic interactions between proteins and ionic surfactants. In this context, proteins which have to be determined and quantified keep their native form, ensuring their migration as expected and thus a good reading of test results.

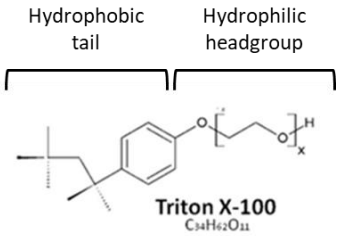


Figure 19. Triton™ X-100 structure

In the same way, to ensure molecules solubilization, detergents have to present an HLB (Hydrophilic-Lipophilic Balance) comprised between 13 and 15. HLB, the proportion between the weight percentages of hydrophilic head and the lipophilic tail in a surfactant molecule is an indication of the behaviour that may be expected from a surfactant. An emulsifier that is lipophilic in character is assigned a low HLB number and an emulsifier that is hydrophilic in character is assigned a high number. The midpoint is approximately ten and the assigned values have ranged from one to forty[34]. So, as HLB factor plays a key role in the protein solubilisation, the detergent which will have been selected will ensure the solubilization and the stabilization in the gel buffer and other reagents solutions of proteins to be identified and quantified.

2. Wettability and optimization of the test sensitivity

As explained previously, in order to ensure a continuous spreading of the proteins to their diffusion limit point, the solution in which the sample (and all the reagents added during the test) is diluted has to offer a surface tension ensuring the absence of obstacles to the movement of proteins, generated for example, by the formation of menisci or air bubbles within the gel.

[34] Gadhave, Determination of Hydrophilic-Lipophilic Balance Value, International Journal of Science and Research, Volume 3 Issue 4, April 2014.

Moreover, performing an enzymatic reaction between an immunoglobulin (Ig) and an anti-Ig in a plastic support cell requires the implementation of certain physical conditions. Indeed, in a liquid medium, it is necessary in this case too, to limit the appearance of bubbles or menisci that could reduce the access of the anti-Ig to the immunoglobulin, for example. Thus, by varying the surface state of the plastic surface, it is possible to reduce the reaction variability and thus improve the sensitivity performance (***Fig. 20***).

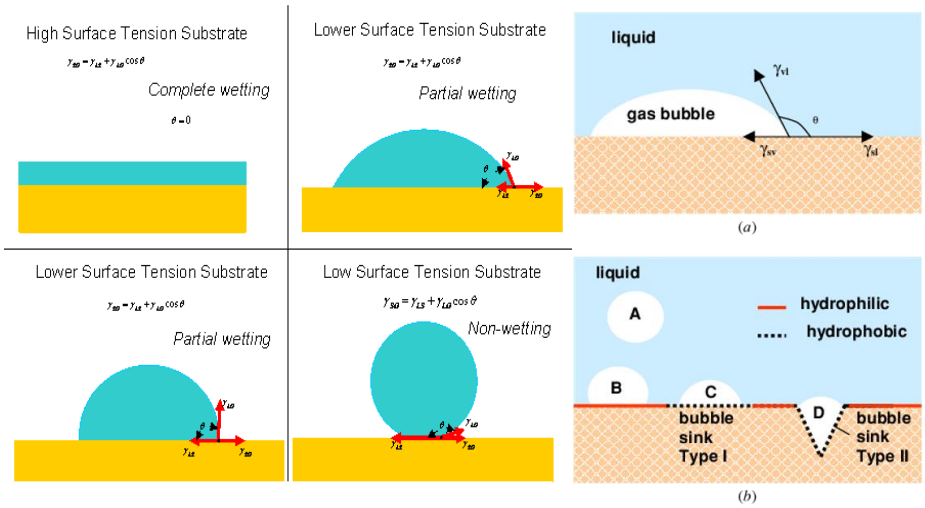


Figure 20. Reaction efficiency related to surface tension

In this purpose, Triton™ X-100 shows excellent wetting properties, based on adequate surface free energy, contact angle, adhesion tension, and liquid-solid interfacial tension[35,36]. In this context, Triton™ X-100 offers the medium conditions required for very sensitive and support-dependant reactions.

3. Continuity between electrophoresis elements and solutions and improvement of the test sensitivity

Thanks to wettability properties offered by Triton™ X-100 and the perfect distribution of the solutions within the device, the continuity between elements constituting the test (strips, gel support, electrodes) is perfectly ensured, thus providing a continuous and homogeneous migration flow from one element to another. In fact, it will be appropriate to use for all the solutions concerned the same detergent or at least detergents of compatible physicochemical properties, in particular viscosity indexes, state temperatures, CMC and HLB regarding their concentrations in the medium. This compatibility must also make it possible to eliminate the different detergents according to identical conditions and to avoid the

[35] Zhang C.-H. and coll. Wettability of Triton X-100 on Wheat (Triticum aestivu) Leaf Surfaces with respect to Developmental Changes, Acta Physico-Chimica Sinica ***2017***, 33(9), 1846-1854.

[36] Today, all chemical suppliers present Triton X-100 as a wetting agent.

presence of residual traces of one of them which may disturb the reading of the final result.

That's the reason why only Triton X-100 is used in the different solutions distributed in the IVD device and concerned by Use-1. In Uses-1, -2 and -4 concerned by this AfA, other detergents (Triton™ X-405 and Nonidet® NP40) are also employed: their selection is based on such criteria.

As a summary, the main functional properties sought-after for the test efficiency during the analysis by downstream-users in view of ensuring the reading reliability and accuracy are the following:

PROPERTIES REQUIRED DURING THE ANALYSIS BY END-USERS	OPTIMIZED PERFORMANCE
Solubilization of proteins to be determined	**Sensitivity**
Stabilization of proteins to be read	**Sensitivity** **Reproducibility**
Good distribution of proteins and continuity between device elements	**Sensitivity** **Reproducibility**

Table 10. Summary of properties expected for highly efficient assays by end-users

3.1.2. Analytical and clinical performances

As described previously, HYDRAGEL® assays are conducted through the use of HYDRASYS® automatic instruments.

Specifications regarding analytical performances have been established for the validation of a potential alternative so as to ensure its compatibility with SEBIA and Interlab analysis instruments.

These include:

- Provide the same sensitivity, specificity, reproducibility and resolution than those obtained with 4-tert-OPnEO;
- Offering to reagents and samples a long-term stability (shelf-life) of several months at temperatures of 2 to 8°C or less than -20°C;
- Ensuring a similar reading of results (profile aspect, fractions positioning, …) through the HLB value as explained previously and through CMC which permits to minimize detergent quantities added to the buffer and thus facilitates its removal at the end of the test. Indeed, the expected clinical performances rest in particular on the reproducibility of the image obtained: the electrophoresis profile of normal fractions and major variants, and the positioning of the fractions must indeed be perfectly identical to what is known by the biologist, considering that the slightest difference can be an anomaly of the patient.

So as to be validated, buffers produced with the potential alternative have, *in fine*, to provide the same diagnosis than those currently produced with Triton™ X-100 when tested against plasma, serum, urine or cerebrospinal fluid from actual patients. Therefore, for each HYDRAGEL® test developed, it is thus necessary to evaluate:

- The analytical sensitivity and/or specificity, that is to say, respectively, the detection limit of a monoclonal protein responsible for the target disease and the ability of the test to detect only the desired proteins and thus, to diagnose or to rule out the pathology which is directly concerned;
- The reproducibility inter- and intra-assays established through the migration of three samples on three HYDRAGEL® devices: means, standard deviations (SD) and variation coefficients (CV) are calculated for each sample and each protein fraction;
- The accuracy of the measurement via sample migration of various biological and SEBIA control samples on both the evaluated SEBIA procedure and a commercially available agarose gel device intended for the detection of proteins of interest. In this purpose, the electrophoregrams are evaluated visually for the presence or not of the marke. Regarding the agreement (concordance), results are then expressed through the correlation coefficient which must be closest to 1.

In addition, when tests are applied to clinical specimens, controls must be tested in parallel to:

- Ensure the quality of the reaction mixture (positive control);
- Exclude a false negative due to the presence in the sample of molecules that inhibit amplification (inhibition control);
- Eliminate a false positive result (negative controls).

Several studies have been carried out to determine performance levels of the different kits. As examples, results obtained for the potential diagnosis of two various disorders or pathologies are provided in what follows. These data are integrated to the products notices furnished to customers as performance references.

→ *Gammopathies*

Performance study is relative to the analysis of serum samples resulting in the separation of the five usual fractions: albumin, α1-globulin, α2-globulin, β-globulin and γ-globulin. Only results concerning albumin are reported[37]:

[37] For other fractions, please refer to the corresponding HYDRAGEL® Protein(e) package insert.

HYDRAGEL® PROTEIN(E)		RESULTS
SENSITIVITY		**0.17 g/L**
REPRODUCIBILITY INTRA-ASSAY*	**MEAN (%)**	**65.8-70.4**
	SD	**0.2-0.7**
	CV (%)	**0.3-1**
REPRODUCIBILITY INTER-ASSAY*	**SD**	**0.2-0.9**
	CV (%)	**0.3-1.4**
ACCURACY*	**Correlation coefficient**	**0.983**

Table 11. Performances of HYDRAGEL® assays for proteins separation in alkaline buffer conditions from human serum or urine samples[38] *(*: Albumin values)*

→ ***Serious liver diseases: HYDRAGEL® ISO-PAL assays***

Different serum samples were electrophoresed using HYDRAGEL ISO-PAL K20 procedure. More particularly, the samples include normal serum, serum with pathological intestinal fractions (I1 and I2) and serum with elevated liver fractions (L1 and L2). Only results relative to the pathological L1 fraction is reported in Table 11:

HYDRAGEL® ISO-PAL K20		RESULTS
SENSITIVITY		**3.0 IU/L**
REPRODUCIVITY INTRA-ASSAY* (3 serum samples tested)	**MEAN (%)**	**40.3-59.7**
	SD	**0.6-1.4**
	CV (%)	**1.0-2.3**
REPRODUCIVITY INTER-ASSAY*	**SD**	**0.3-2.0**
	CV (%)	**0.6-8.5**
ACCURACY*	**Correlation coefficient**	**0.991**

Table 12. Performances of HYDRAGEL® assays for identification and quantification of alkaline phosphatase in human serum in view of diagnosing liver diseases[39] *(*: L1 liver fractions values[40])*

3.1.3. Risks for human health and the environment

To be valid, a potential alternative has to present a lower level of risk for human health and the environment than that of Triton™ X-100. Candidate alternatives have not to be classified as a SVHC under the REACh regulation.

[38] See corresponding SEBIA package insert: HYDRAGEL 7/15/30 Protein(e), ***2009***.

[39] See corresponding SEBIA package insert: HYDRAGEL ISO-PAL K20), ***2004***.

[40] For other fractions, please refer to the corresponding HYDRAGEL® ISO-PAL K20 package insert.

3.2. Market and business trends

3.2.1. Use of 4-tert-OPnEO

The quantity of 4-tert-OPnEO used under Use-1 is provided in Table 12 below:

kilograms	2015	2016	2017	2018	2019*	2020*	2021*	2022*
Triton™ X-100 SEBIA	**77.293**	**52.727**	**50.952**	**67.785**	**75.24**	**83.52**	**91.87**	**100.14**
Triton™ X-100 REXOR	**0.756**	**0.253**	**0.539**	**0.365**	**0.41**	**0.45**	**0.49**	**0.53**
TOTAL Triton™ X-100	**48.049**	**52.980**	**51.491**	**68.15**	**75.65**	**83.97**	**92.36**	**100.67**

Table 13. Consumption of Triton™ X-100 over the 2015-2018 period under Use-1, in kilograms.
(= Forecasts)*

These forecast annual consumptions are based on the further elements:

- **For SEBIA**: a 11% forecast annual increase, based on the current SEBIA growth, is applied on Triton™ X-100 consumptions planned for 2019 and 2020. In addition, from 2021, SEBIA, by engaging in the substitution of Triton™ X-100, envisages a decrease in its consumption of 1% per year, up to 9% in 2022. Indeed, it should be noted that **the business model of Sebia is based on a growth of 8% per year, calculated on the basis of investments and current developments of the Company**. In this context, at less than 8%, the viability of the Company would no longer be ensured. For this reason, consumption, until substitution, will continue to follow this maximum growth rate of 8%;
- **For REXOR**: the forecast annual consumption increase follows the same corresponding annual growth as SEBIA.

In conclusion, the average 4-tert-OPnEO consumption of SEBIA over the 2015 – 2018 period, is 62.190 kg per year. For REXOR, the average consumption of the substance over the 2015-2018 period is 0.478 kg per year. Consequently, the average 4-tert-OPnEO consumption of the two entities is 55.17 kg per year.

3.2.2. Business trends

To date, SEBIA is the leader in the European market with nearly 95% market share in electrophoresis. Growth on this technique is of the order of 6%. The flagship ranges are Protein electrophoresis (20% of sales), followed by Immunofixation (25%) and immunotyping (6%) with a strong growth in the diabetes market (6%).

In order to pursue its growth, the business development strategy of SEBIA is based on the following goals:

- **Consolidate its leadership in Myeloma** in proposing integrated solutions enabling fast conversion from Gel to Capillary technology and through high medical value information provided by powerful software solutions enabling better patient management;
- **Develop its position in Diabetes & Haemoglobinopathies** in accompanying customer in their transformation journey to full lab automation in consolidated markets or markets in phase of consolidation;
- **Diversify its product portfolio** in adding and promoting high value specialty test leveraging SEBIA's technologies.

The business model is therefore based on a B-to-B system with a product offering that includes the supply of all products, services and associated services integrated into customer contracts. SEBIA offers its products and services in the context of competitive bidding, negotiated contracts with sales contracts that deal with direct sales, provision of instruments and billing of the cost per result. Thus, SEBIA is committed to providing optimal and consistent performance solutions, in order to best meet the requirements of customers who are themselves subject to extremely high requirements.

3.3. Human health impacts of the "applied for use" scenario

4-tert-OPnEO was included on Annex XIV of REACh for the impacts on the environment of its degradation products; as a consequence, impacts on human health are excluded of the context of the present application.

3.4. Impacts on the environment and monetised damage of the "applied for use" scenario

A direct causal link between exposures to 4-tert-OPnEO and impacts on the environment is difficult to establish in a robust manner, especially in the case of the present application for Authorisation where substance releases are already reduced to a minimum (near zero).

However, and in view of providing a comprehensive understanding of the situation, several elements of context will be provided in what follows, that pertain to:

- The **environmental context** of the impacts – hydrographical situation around SEBIA' sites and characterisation of the receiving medium;
- Elements of **qualitative environmental impact characterisation** – use and non-use value.

4-tert-OPnEO is used under Use-1 in two French sites, Lisses (for SEBIA) and Paladru (for REXOR). As the same substance is used under other Uses presented in

this AfA in the site of France too, environmental impacts and monetised damaged will only be presented for the REXOR's site, considering that those relative to the site at Lisses is described in the dossiers relative to Uses-3 and -4.

3.4.1. Management of aqueous waste relative to Use-1: discharges evaluation, collection and treatment in the context of the "applied for use" scenario

In order to reduce the amount of liquid and solid waste generated by its activities, REXOR has already implemented numerous measures. As an evidence and regarding the current corresponding Waste Regulation, soiled solid waste and the majority of effluents containing 4-tert-OPnEO are collected and taken in charge by approved processing systems.

Soiled solid waste are usually obtained from R&D activities and thus contain negligible quantities of 4-tert-OPnEO. Nevertheless, all the samples which production involves the use of agarose gel containing 4-ter-OPnEO, are incinerated.

Concerning liquid waste, effluents containing 4-tert-OPnEO come essentially from recovery of rinsing water from coating lines and corresponding equipment. Indeed, after a preliminary collection of rinsing waters then treated through an incineration process, a second rinsing step is carried out, releasing potential residual quantities in the collective network.

In addition, during the process of gel coating, Triton® X-100 is quite entirely transferred on the plastic support and the remaining solution containing 4-tert-OPnEO is sent back to SEBIA without handling or switching the agarose gel from the initial container to another one.

So, today, all the water effluents generated during the cleaning of glassware and other containers are treated and incinerated. Moreover, excluding potential residual quantities of 4-tert-OPnEO coming from a second rinsing step of the coating line equipment, it can be considered that releases will be extremely low and should not contribute to an eventual contamination of the immediate environment. As a probe of assessment, they will be evaluated with measurements as close as possible from where potentially releasing processes occur.

3.4.2. Environmental context of the impacts

3.4.2.1. Direct environment of REXOR facilities

The site is located in Paladru, in the department of Isère (Auvergne-Rhône-Alpes region) in a very preserved environment. This commune is mainly known for its lake. Inherited from the ice ages, the Paladru Lake is the largest lake in the Isère region. An

ancient human presence is the subject of fruitful archaeological research. Today leisure activities coexist with more traditional fishing.

Moreover, the reedbeds of the lake are protected by a prefectural decree that prohibits any penetration and destruction of the reeds: to date, Paladru is classified as a ZNIEFF[41, 42], as the testimony of the exceptional nature reserve of the region.

In this context, the region has a large network of rivers and lakes: in particular, the Isère River, Paladru and Lépin Lakes are widely exploited by the department. The site is at less than 800 m from the Paladru Lake (***Fig. 21 & 22***).

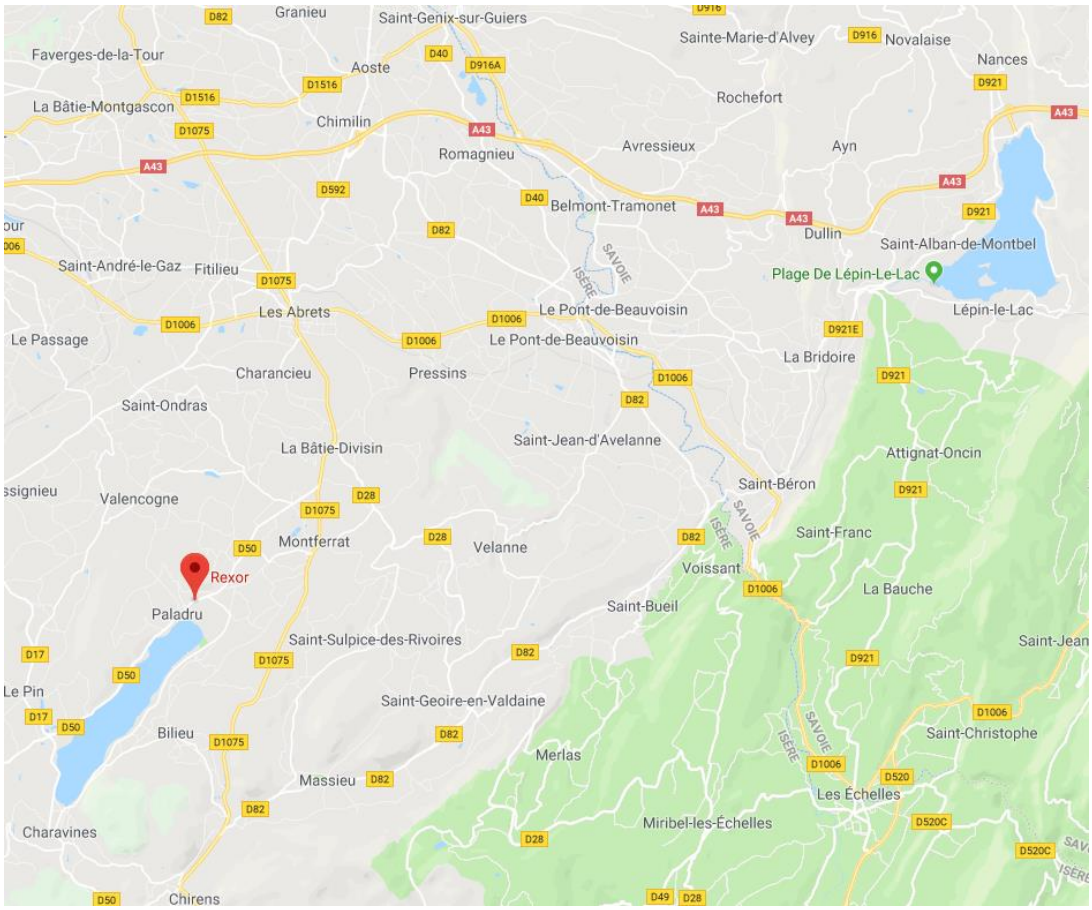


Figure 21. Location of the REXOR site and of closest watercourses

[41] Source : Inventaire National du Patrimoine Naturel – National Inventory of the Natural Heritage - inpn.mnhn.fr

[42] ZNIEFF : Zone Nationale d'Intérêt Ecologique, Faunistique et Floristique - Natural Areas of Ecological Interest for Fauna and Flora.The ZNIEFF aim to identify and describe areas of the territory particularly interesting ecologically, participating in the maintenance of major natural balances or constituting the living environment of animal species and rare plants, characteristics of the regional natural heritage.

Figure 22. Location of Rexor's facilities near the Paladru Lake

The Isère River, located in the south-east of France, is one of the most important tributaries of the Rhone and measures 286 km. It takes its source in the Alps and flows into the Rhone a few kilometers north of Valence.

The Paladru Lake is located at an altitude of 492.40 meters NGF. It extends over the municipalities of Paladru, Montferrat, Bilieu, Charavines and The Pine. Its length is 5.300 km, its average width of 800 meters (1 km at the widest) and its depth is 32 meters in the middle. Its area is 392 hectares, which makes it the fifth natural lake in France after the Geneva Lake, the Bourget Lake, the Annecy Lake and the Aiguebelette Lake. It is fed mainly by precipitation. Its outlet to Charavines is the Fure River which flows into the Isere. A system of valves makes it possible to regulate the flow of water at the exit of the lake according to the needs of the factories established on the course of the Fure. In the second half of the 19th century, this river fed paper mills, steel mills, cutlery, weaving, etc ....

The total capacity of the lake is 97 million m^3. The total renewal time of the lake water is estimated at 3.6 years. The lake level varies. The amplitude of the variations can reach 2.50 m, which is not without consequences on the flora, the fauna and the riparian installations.

The lake's fauna is one of its great riches. The marsh of La Véronnière in the north, classified "protected natural area", is one of the most beautiful bird sanctuaries in the Dauphiné: grebes, herons, kingfishers, ducks, teals, scoters, coots, swans find shelter and food, to name a few species. The aquatic fauna is not less rich: pike, carp, perch, tench, lavarets, crayfish, Arctic char, trout, etc ..., make the Paladru Lake a popular place for fishermen. There are also water molds (anodonts) that are highly toxic. On the other hand, they filter the water and help purify it. The

aquatic flora includes many species of plants, some of which are essential to the life of the lake and the wildlife that inhabits it. For example, the reeds participate in the natural sanitation of the lake and shelter the nest of coots. Water lilies beautify the lake during flowering. Underwater herbs help small fish protect themselves from their predators.

Nevertheless, if the department of Isère has a very dense hydrographic network, it is in addition to the rainfall and topographic characteristics at the origin of a very strong sensitivity of the territory to the floods.

Indeed, the major part of the Isère area is located in a potential flood zone and is considered as a TRI (Territoire au risque Important d'Inondation – Territory with a potential significant flood risk) according to some territory specificities (dangerousness of the phenomena, hydraulic coherence, demographic or seasonal pressure, socio-economic characteristics, ...). In this context, more than 50% of the population and jobs in the urban unit are at risk of flooding, mainly in case of ruptures of dikes, of Isère, Drac, Romanche, Fure and Morge Rivers. Nevertheless, the zone upon which REXOR site is located is not directly concerned by this risk (***Fig. 23***).

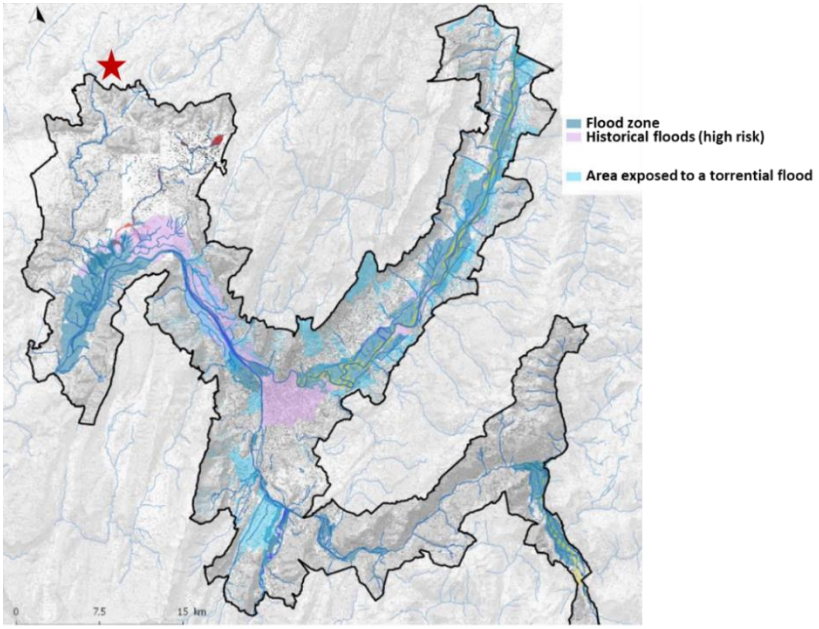


Figure 23. Extent of potential water overflows in the REXOR site zone[43]

★ *REXOR facilities*

The region thus benefits from both a natural risk management and a policy of monitoring of the ecological and chemical state of its environment. In the context of the Water Framework Directive, the management of this hygrometric network is

[43] Stratégie locale de Gestion du Risque Inondation Territoire à Risque Important d'inondation (TRI) de Grenoble – Diagnostic Territorial v 8.0, ***July 2017***.

carried out at a "catchment area" level, which is in this case, the “Rhône-Méditerranée basin”.

Figure 24. Delimitation of the Rhône-Méditerranée catchment area[44]

★ *REXOR facilities*

Key figures for the Rhône-Méditerrannée catchment area include:

Catchment area surface	127, 000 km²
Population	15.5 million inhabitants
Typology of territories	- Agricultural area 27 % - Forests 33 % - Herbal surface 14 % - Artificial surface 24 % - Water surface 2 %
Seafront lenght	1000 km

Table 14. Rhône-Méditerranée catchment area key figures[45,46]

The management of the watercourses of the basin is carried out more particularly by the Rhone-Mediterranean Water Agency. It is currently planned in the Master Plan for Water Management and Management (SDAGE) of the Rhône-

[44] http://www.rhone-mediterranee.eaufrance.fr/presentation.php

[45] Final environmental evaluation report – Rhône-Méditerranée SDAGE, 2016-2021: http://www.rhone-mediterranee.eaufrance.fr/docs/sdage2016/docs-officiels/20151221-RapportEnvironnemental-2016-2021.pdf

[46] Bassin Rhône-Méditerrannée – Etat des lieux ***2013***.

Méditerranée basin: in this framework, the ecological chemical status of water bodies is regularly evaluated.

3.4.2.2. Ecological and chemical status of water

The Rhône-Méditerranée basin includes many water bodies: surface water (rivers, lakes, …), groundwater and coastal waters. The monitoring of the water quality carried out within the framework of the SDAGE shows, since 2010, a global improvement of the water quality for the most degraded environments but without reaching the good state (***Fig. 25-a***). Thus, more than half of the surface water bodies are in good ecological condition, and a large majority present a good chemical status according to the inventory carried out in 2013.

It's the case of the Paladru Lake for which ecological status (***Table 15***) is considered "Good". The chemical status is quite good too, considering that the only declassing substances are benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene. Nevertheless, considering the 18 waterbodies located on the territory, the lake of Paladru is part of the 5 water plans presenting risks with regard to the achievement of the good state - or the good potential - ecological, in particular in terms of diffuse pollution in pesticides. Diffuse pollutions with eutrophying effects (mainly phosphorus, and secondarily nitrogen) also constitute a risk for the lake. These are linked in particular to the presence of intensive crops.

Parameter	Phythoplancton	Macrophytes	Turbidity	Dissolved oxygen	Nitrogen	Phosphorus	Specific pollutants
Status	Good	Good	Very Good	ND	Medium	Very Good	Good

Table 15. Ecological status of the Paladru Lake[47]

In parallel, 80 % of groundwaters are in good condition, in ecological and chemical terms (***Fig. 25-b***). In a same way, 70 % of coastal waters are in good ecological and chemical states (except ubiquitous substances) according to the inventory of 2013. Including the ubiquitous substances, 63% of the coastal waters present a good quality. Coastal waters are under the direct influence of the contributions of the terrestrial catchments but also of the human activities on the littoral and at sea (***Fig. 25***).

[47] http://sierm.eaurmc.fr/surveillance/plans-eau/index.php

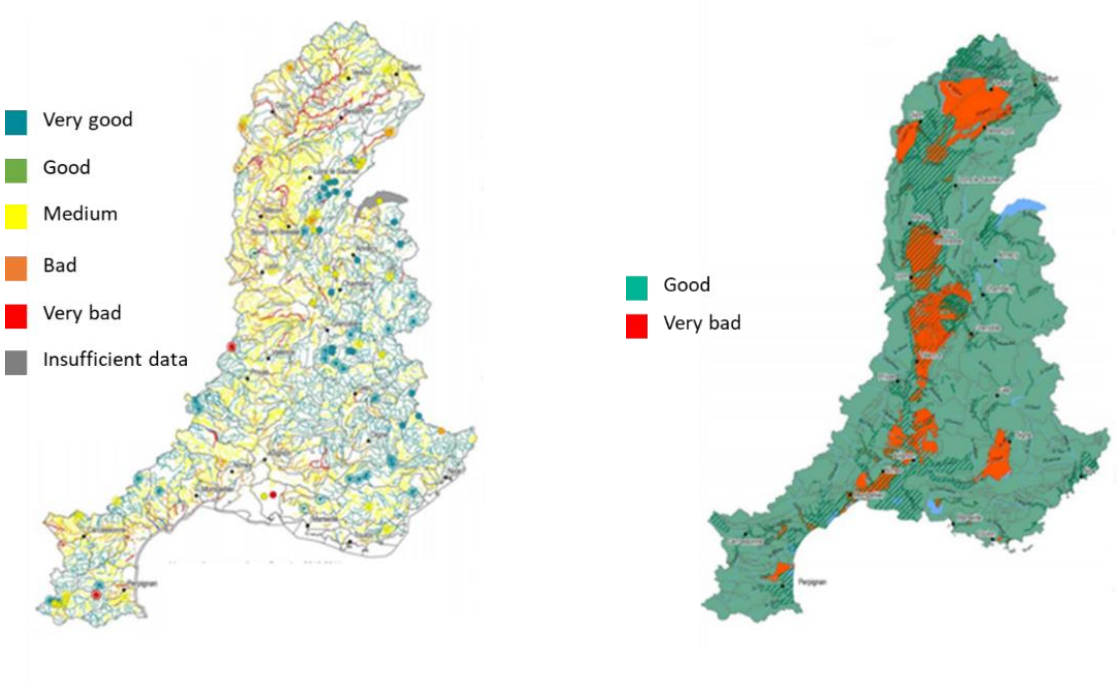


Figure 25. Chemical state of superficial water and groundwater for the Rhône-Méditerranée catchment area (*surface waters and groundwaters, respectively*) [43]

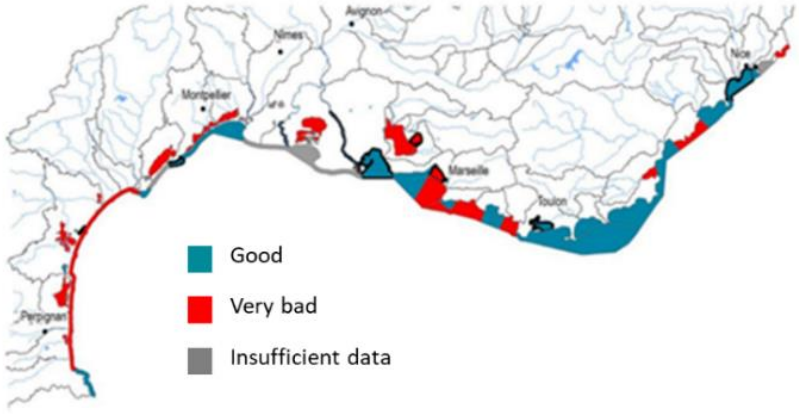


Figure 26. Water chemical quality of Rhône-Méditerranée coastal waters

So, the global ecological and chemical status of the basin waters is quite "Good", as at the level of the Rhône-Alpes region, for which results are similar (***Fig. 27***).

Figure 27. Status of waterbodies in the Rhône-Alpes region[48]

As proof of the quite good water quality, the quality of the fish population, which is a suitable indicator, remains relatively stable in the Rhône-Mediterranée basin. Measurements made on 346 reference stations distributed throughout the area show a fish index which seems to improve (***Fig. 28***).

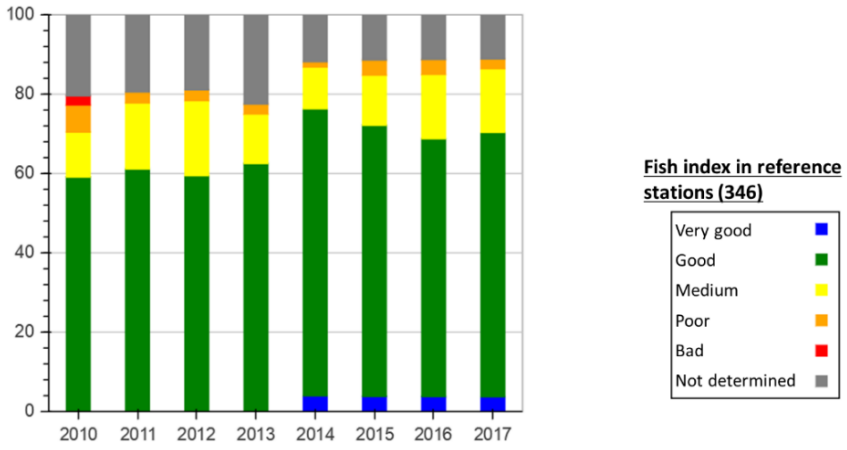


Figure 28. Fish index in Rhône-Méditerranée basin[49]

[48] Qualité des cours d'eau dans la région Rhône-Alpes, Direction régionale de l'Environnement, de l'Aménagement et du Logement Rhône-Alpes, ***2015***.

Overall, 54% of stations have a "good" to a "very good" fish status. These states are mainly observed on basin head sectors, upstream of the anthropized plains.

At the local level of the Paladru Lake, the fish index is improving. Nevertheless, there are still functional problems (deoxygenation, excess of organic matter, water level fluctuations) that still constrain biological and fish production. Simultaneously, the management of fish (especially by spilling fish populations) masks the difficulties encountered by certain species in completing their life cycle (Arctic char, pike, cyprinids, etc.) and it is difficult to quantify and qualify with precision the range of the improvement.[50]

Thus, the immediate area of the REXOR site has a good piscivorous profile which proves a global good water quality.

3.4.2.3. Substances measured in the aquatic environment of REXOR

Regular sampling campaigns carried out in Isère areas and in the heavily industrialized area along the lower Rhône thus made it possible to identify the substances at the origin of such a chemical state and which are now considered to be priorities at European level.

Indeed, unlike the ecological state that results from point and diffuse pressures generated by the rejection of several hundred substances, the chemical state is evaluated according to a risk management approach involving a list of 41 substances and for which it is necessary to engage a risk reduction approach.

The alkylphenols (nonylphenols, octylphenols), which are degradation products of 4-tert-OPnEO, are part of this list and thus constitute a point of attention within the framework of the Water Framework Directive. Measured in quantities large enough to generate a high risk on nearly 19 water bodies, these substances are very present in the basin, especially in areas located downstream of chemical industrial activities. The inventory carried out within the framework of the SDAGE between 2010 and 2013, and based on the calculation of the rejected flows of 1,200 ICPEs and more than 290 treatment plants distributed throughout the basin, thus showed an average annual flow of nonylphenols and para-tert-octylphenols of 197,135 grams per year and 12,621 grams per year, respectively.

So, these substances contribute to the quantification of the global chemical state of the area but in low quantities, in comparison with other substances such as dichloroethane (2, 311 kg per year), tetrachloroethylene (968,421 kg per year), nickel (5,089 kg per year) or lead (1,615 kg per year).

[49]http://www.observatoire-des-territoires.gouv.fr/observatoire-des-territoires/es/indice-poissons-riviere

[50] http://www.paladrupeche.fr/wp-content/uploads/2018/02/lac-pala-diagnose06.pdf

In parallel with these measurements, sediment samples taken at various stations in the Isère, Drôme and Rhône regions revealed the presence of alkyphenols in quantities considered both "undesirable" and dispersive: nonylphenols were quantified in 14 stations (i.e. 7 % of selected sites) and 4-tert-octylphenol was measured in 18 stations (i.e. 5% of selected sites)[51]. In stations near the REXOR site, octylphenols have not been quantified.

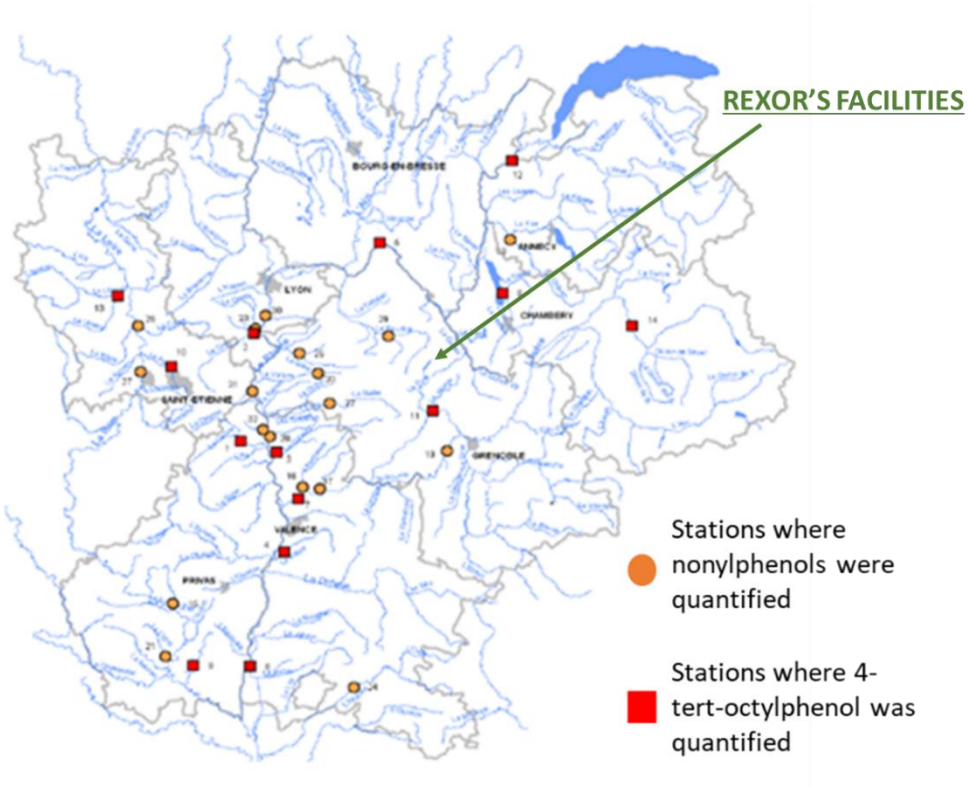


Figure 29. Quantification of alkylphenols in the Rhone-Alpes region

These region-wide results are confirmed on a smaller scale: annual assessments of the ecological and chemical states of the Isère River, published by the Rhône-Méditerranée-Corsica Water Agency report[52] :

- A good to a very good ecological state over the last three years, with respect to the main reference parameters (oxygen balance, temperature, nitrogen and phosphorus nutrients, specific pollutants, …)[53] ;
- A "good chemical status" not achieved since 2015. Micro pollutants, like polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs)

[51] Report « Micropolluants dans les sédiments de la région Rhône-Alpes – Données cours d'eau et plans d'eau 2006-2011 » – Service Ressources EnergieMilieux et Prévention des Pollutions, ***2013***.

[52] http://www.rhone-mediterranee.eaufrance.fr

[53] In accordance with the decree of July 27, 2015 : http://sierm.eaurmc.fr/surveillance/eaux-superficielles/documents/arrete_evaluation_etat_eaux_de_surface_27-07-2015.pdf

> and pesticides have been identified in large quantities in Isère tributaries. Since 2011, the RSDE 2 measurement campaign has provided results showing the insignificant presence of octylphenol and significant quantities of nonylphenols at the outlet of treatment plants, based on the calculated annual flows and the acceptability conditions of the receiving environment. Urban discharges are usually the primary sources of these substances.[54]

In conclusion, if the ecological potential of the Rhône-Méditerranée basin remains relatively high today, the good chemical state is not reached. The results are identical in the immediate environment of the REXOR site where actually, nutrients and pesticides are the main declassing substances. At the region level, 4-tert-OPnEO type derivatives were also measured but in amounts considered insignificant and which cannot be considered as contributing to the current chemical state. In the special case of the Paladru Lake, alkylphenols were not measured and quantified. Finally, in general, throughout the Rhône-Alpes region, the quantities of this substance are low, and its distribution not diffuse, even if its concentration is a little more important near major industrial sites.

In summary, the alkylphenols are not in sufficient quantities to participate in not reaching the good chemical state.

As a result of this study, the Rhône-Méditerranée catchment area shows a pollution mainly related to industrial hydrocarbon releases.
Octylphenol is part of this pollution. Nevertheless, in comparison with other substances, it was measured in the Isère region in amounts considered insignificant. Moreover, in the immediate area in which REXOR site is located, contamination measurements have shown no trace of these substances, demonstrating that 4-tert-OPnEO discharges resulting from various – mostly domestic and minority industrial - activities can be considered insignificant and without contribution on the ecological and chemical status of the direct environment.
Consequently, it can be argued that releases from REXOR's activities currently close to zero, will have no impact, too.

[54] « Bilan des flux de métaux, carbone organique et nutriments contenus dans une rivière alpine: part des rejets urbains de l'agglomération de Grenoble et apports amont (Isère et Drac) » S. Dutordoir – Thesis from Université de Grenoble, ***2014***.

4. SELECTION OF THE "NON-USE" SCENARIO

A significant work of research and testing of potential alternatives to Triton™ X-100 in the context of Use-1 was carried out by SEBIA.

As a result of this initiative, one potential alternative has been identified that is foreseen to meet the functional requirements laid out in the previous section.

This potential alternative, however, has yet to undergo further empirical investigation, quality validation and industrialisation; it will therefore not be available to SEBIA before the sunset date of 4-tert-OPnEO.

4.1. General presentation of SEBIA's change management process

"Change control" applies to any change in the life cycle of a product that has a direct or indirect impact on the quality, efficiency or safety of the product and associated systems.

This procedure applies to any changes made to:

- The design
- Existing product (including labelling or packaging)
- A manufacturing process (the process as well as the material)
- Infrastructure
- The documentation
- Software applications
- Processes (Management System)
- Purchase data

All changes do not require a change control procedure before approval and implementation. Low risk / impact on product or customer changes are not required to be handled under this procedure.

Main steps of this process are the following:

1. **Change request**

   In this step, the consequences of the change on the functions, performance, usability, product safety and applicable regulatory requirements for the device and its intended use are reviewed and evaluated.

   This step is performed via the Internal Change Validation (ICVP) process (***Fig. 30***).

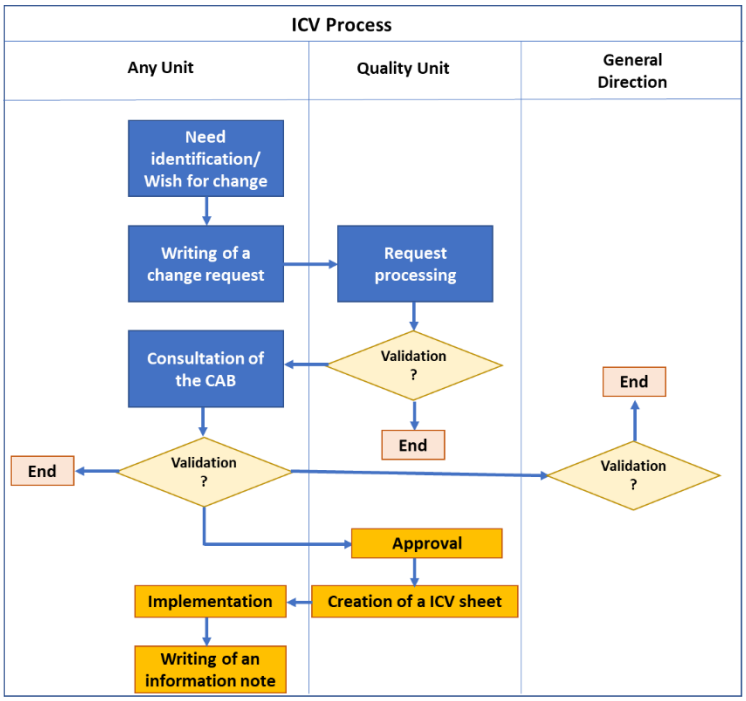


Figure 30. Internal Change Validation Process

2. Processing of the request by the Quality department

The request is processed by the Quality department, which performs a filtering. It defines CAB (Change Approval Board) members for the relevant application. The CAB then evaluates the feasibility of the modification. This multifunctional group is composed of experts selected according to the change to be analysed (quality assurance, regulatory affairs, marketing, etc.). It evaluates the request and determines the action plan. Each CAB member renders an opinion after conducting an analysis of the impact of change on their "area of expertise" and must ensure that, once the modification is made, the product remains of the desired quality and conforms to the approved specifications.

3. Decision and change implementation

Depending on the assessment, the change request may be approved or denied. In some cases, the request can be forwarded to the General Management who can approve or reject this request. In the event of approval of the request, an amendment to the specifications is carried out and the steps of the design process are carried out.

The modification is thus reviewed, verified and validated, when appropriate before final approval. All impacted design documents are then updated.

In the case of a "substantial" change, it is necessary to notify this change to the notified body. In a same way, where required by international regulations, substantial changes to a device approved by a regulatory authority shall be communicated to that authority.

4.2. Substitution initiative

4.2.1. Elements of context

The substitution of Triton™ X-100 in the production and use of the electrophoresis kit takes place in a complex commercial context, relative to the market covered by SEBIA.

In particular, HYDRAGEL® solutions are used:

- By various customers (universities, laboratories and hospitals);
- By private companies and public institutions and organizations;
- In many countries in Europe and outside Europe.

It results then in a hyper diverse and complex operating environment whose constraints are different.

So, according to the diversity of applications and markets covered, the substitution of Triton™ X-100 is a technical, commercial and regulatory issue: beyond the difficulty of identifying an alternative adapted to these numerous applications, the system will have to be revalidated by all the customers of HYDRAGEL® products on the basis of criteria specific to their applications. At the same time, the regulatory compliance of the new medical device will have to be implemented in the various countries where these products are marketed.

→ ***HYDRAGEL® range validation: a key point in the substitution initiative***

The substitution of 4-tert-OPnEO in the HYDRAGEL® system necessarily implies the revalidation of corresponding solutions and associated equipment, depending on the applications concerned.

Indeed, the performance requirements will first relate to the ability to immobilize lipoproteins within the gel and thus to avoid a misinterpretation of the result linked to a bis-albuminemia reading. Thus, a good separation of lipoproteins from albumin is essential for the successful reading of the test results. This separation efficiency depends on the interactions between lipoproteins and detergent molecules but also on the good migration of proteins to be determined, ensured by the solubilisation of native proteins in the gel. The detergent has thus a crucial role in the separation process of proteins of interest.

Once these buffers are validated, the HYDRASYS® machines (including corresponding hardware, firmware, software and associated protocols) may also have be readapted to the new electrophoresis assay, as any change likely leads to the modification of some parameters.

To illustrate this point, the substitution of one component, like 4-tert-OPnEO, may lead to a new global buffer formulation, requiring the adaptation of the electrophoresis protocol and/or assay conditions such as concentrations, pH, or addition of various additives. This could also involve the adaptation of the process conditions (temperature and time).

→ *External validation of the new electrophoresis solution*

As explained previously, this electrophoresis system uses an interdependent series of buffers to ensure the migration and thus the separation of proteins of interest from an extensive range of clinical specimens and for a diverse range of *in vitro* diagnosis assays and R&D applications.

Consequently, a complete performance revalidation on a large and highly diverse range of specimen's and applications must be done internally but also externally during clinical trials on various IVD applications.

Thus, maintaining identical performance is crucial to securing these markets. Nevertheless, given the complexity and diversity of electrophoresis assays and applications, the likelihood of substituting Triton™ X-100 while maintaining the same performances across all the user application seems remote and even if a reasonable substitute was found, it would still lead to notification to the market and subsequently serious user disruption due to adaptation, revalidation and registration or forced changes.

→ *Regulatory issues in the validation of the new electrophoresis products*

According to the requirements regulating medical diagnostic devices, the change of detergent will have regulatory impacts. At European level, any in vitro diagnostic medical device placed on the market or in use must have a CE-IVD marking. This CE-IVD marking may be affixed to an in vitro diagnostic medical device only if it complies with the essential requirements and has been the subject of the evaluation procedures applicable to it. All of the essential requirements and conditions for compliance are described in Regulation No. 2017/745 of 5 April 2017 on medical devices repealing Directives 93/42 EC and 90/385 / EEC. It was published in the Official Journal of the European Union on May 5, 2017 .[55]

Thus, in addition to the CE marking, this regulation has a major impact on the sector and on all of its stakeholders by governing in particular:

- Placing on the market and movement of the authorized devices;
- Economic operator obligations;
- Traceability;
- UDI (Unique identification of devices);
- Device classification;
- Conformity assessment;
- Evaluation and clinical investigations;
- Post-market surveillance including complaint identification and management;
- …

[55] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0746&from=EN

So, in the case of the modification of a component or a parameter, this provision necessarily implies the reassessment of the conformity of the device concerned and its regulatory requalification. The substitution of the detergent will likely require a new registration of the IVD test depending upon the jurisdiction.

In addition, this regulatory obligation will have to be implemented in all the countries where the system is marketed, namely in Europe but also outside Europe (Brazil, China, Russia, Canada, …) where regulatory processes require various time delays for registration inducing, consequently, the need for SEBIA to anticipate safety stock of products before to implement the product with new formulation.

For example, it is the case of the American FDA[56] regulation on medical devices. In this framework, an Authorisation of production and development of the device has to be granted to the Applicant. In this context, the FDA exercises general controls under standardized procedures. These controls include the following requirements:

- The FDA registration of the establishment and all products marketed in the US market;
- Compliance with the labelling requirements of the CFR (Code of Federal Regulations);
- The design and manufacture of medical devices in accordance with Good Manufacturing Practices (GMP) as described in section 520 of the FD & C Act.

After the marketing approval by the FDA, which sometimes imposes obligations to be complied with during the marketing phase (additional studies to be carried out, restrictions on the sale, distribution or use of the device, etc.), the Agency requires the application of a system of quality standards and continuous monitoring through regular reports in case of malfunctions or incidents.

In conclusion, these different elements - possibility of substitution, customer change acceptance, client adaptation period and validation, impacts and duration related to the different regulatory procedures - are key points in the evaluation of the review period required in the framework of the HYDRAGEL® products range integrated in Use-1.

[56] FDA website - http://www.fda.gov

4.2.2. Substitution initiative

SEBIA substitution initiative was structured as a seven-phase project:

- **Phase 1** – Screening of surfactants;
- **Phase 2** – Pre-selection of best candidates and R&D feasibility study;
- **Phase 3** – R&D validation of the selected surfactant: optimization tests;
- **Phase 4 -** Confirmation of analytical performances of selected surfactants on test batches;
- **Phase 5** – Industrial validation of the substitution and manufacturing of pilot lots
- **Phase 6** – External clinical testing and validation
- **Phase 7** – Regulatory registration
- **Phase 8** - Commercial deployment: system changes, customer management (communication and use revalidation)

The content of these phases is detailed in what follows. In addition to this process, the following constraints will apply:

- The selected candidate will have to be compatible with all alternatives selected for the other solutions. Indeed, in the majority of cases, a kit is concerned simultaneously by three or four uses. Moreover, one of the most fundamental rules of electrophoresis to obtain reproducible results is to ensure the continuity of the chemical species between the gel and the other electrophoresis components, including strips and reagents, as described previously. In this context, each chosen candidate will be likely to fulfil at least one of the requirements of the three other Uses detailed in the context of this AfA and to be complementary of other candidates selected and present in solutions concerned by Uses-2, 3 and 4;
- According to the criticality of these products, full-scale production batches will have to be carried out, which will impact the overall production schedule;
- The substitution will only be possible if there is no interruption to the availability of assays to customers. Indeed, in the special case of Myeloma assays, SEBIA is a “pure player” and a reference supplier of this kind of test. Moreover, as ranges dedicated to Myeloma serve mainly the early detection of the disease, the supply discontinuation is inconceivable. That’s the reason why a significant elapse time will be considered as a market cessation;
- The timeline defined (§5.3.3) requires taking into account the validation of the performance of IVD products which is based on health constraints imposed by the regulations in force: in particular, all stability studies - accelerated stability, stability in real time - must be performed. With a duration of three months and thirty-six months, respectively, the time required for these studies must be included in the overall calculation of the

timeline necessary for the development and marketing of a solution without 4-tert-OPnEO;

- The ultimate validation of the selected solution will then depend on the end-user's qualification.

Moreover, the substitution could have the following subsequent impacts:

- This substitution could divert some end-users from the HYDRAGEL® solution, because of a possible time delay due to the unavailability of a satisfactory alternative solution providing same performances. It could be linked to the inability of SEBIA to substitute the component by another one, regarding parameters like technical feasibility, R&D and industrialization costs, timeline ... So, a delay in timeline of the project could have some public health and market consequences and a huge direct economic impact for SEBIA;
- As all HYDRAGEL® ranges are concerned by Use-1, in the event of failure in the development of an alternative solution with perfectly identical performances, and consequently resulting in the complete loss of the associated market for SEBIA, the economic consequences would be catastrophic and could jeopardize the survival of the company.

Thanks to a bibliographic review, a primary selection of several non-ionic surfactants has been made through a bibliographic research on considering their non-ionic nature, their cloud point, their CMC and the Hydrophilic-Lipophilic Balance (HLB) values (***Table 16***).

DETERGENT	CAS	Molecular Weight	Average aggregation number	CMC (mM)	CLOUD POINT (°C)	HLB
TRITON™ X-100	9002-93-1	625	140	0.24	64	13.4
BRIJ® 35	9002-92-0	1225	40	0,09 mM	**> 100**	16,9
BRIJ® 58	9004-95-9	1122	70	0,08	**> 100**	15,7
DIGITONIN	11024-24-1	1229	60	0,67-0,73	**	0,4
TWEEN® 20	9005-64-5	1200	**	0,059	**76**	16,7
TWEEN® 80	9005-65-6	1310	60	0,012	**65**	15
DODECYL- β-D-MALTOPYRANOSIDE	69227-93-6	510	98	0,15	**	**
OCTYLGLUCOSIDE	29836-26-8	292	84	25	**> 70**	**

Table 16. Pre-selection of detergents[57]

[57] Detergents for Cell Lysis and Protein Extraction (https://www.thermofisher.com/fr/fr/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/detergents-cell-lysis-protein-extraction.html)

More precisely, the selection of these seven detergents results from an iterative approach. Indeed, based on the most commonly used detergents found in literature data, a first round of selection of non-ionic detergents having the closest physico-chemical properties to Triton™ X-100 was made. Non-ionic structures were mainly selected because they are non-denaturing detergents, meaning that they should not impact the target protein structures involved in the test.

Then, the compatibility of these candidates with the global HYDRAGEL® workflows has been anticipated too. Therefore, facing the high number of detergents, a work based on critical physico-chemical factors (CMC, cloud point, …) was carried out. Finally, the HLB (hydrophilic Lipophilic balance) and the CMC seemed to be very discriminating parameters to use to keep the best candidates among this preselection. Thus, based on the hypothesis that the detergent having the HLB and the CMC the closest possible to Triton™ X-100 ones could present similar solubilisation and separation properties, the six detergents mentioned above were identified as the best candidates to substitute Triton™ X-100.

Finally, a regulatory study was made to remove all the substances which are dangerous or very toxic (CMR, BT, …) or which could be integrated in the future Candidate List and then in the Annex XIV. That's the reason why Brij® 58P has been recently discarded from this pre-selection, according to a possible evolution of its regulatory status related to potential "SVHC" properties.

So, following this iterative study, six surfactants could be subjects of further R&D phases. More particularly, Tween® 20 could provide suitable properties, according to SEBIA expertise and recent works concerning the successful replacement of Triton™ X-100 by Tween® 20 in detergent solutions used in the washing of certain electrophoretic components. Nevertheless, the Tween® 20 remains only one track, this substitution carried out being the easiest one to achieve: indeed, the only function expected was the washing property, essentially common to all detergents, by definition.

Consequently, these surfactants will undergo a series of preliminary testing in order to establish a first functional performance list and then to identify the most promising options.

→ *Phases 2 to 4 – Pre-selection of best candidates: first R&D feasibility study, optimization tests and performances validation*

To date, the feasibility tests have not started yet due to lack of internal resources. Recruitment of staff is currently underway. Nevertheless, a road map has already been defined, according to steps and a precise timeline. This organization scheme also takes into account the other functions of the substance covered by this AfA through Uses-2, 3 and 4, because of the absolute necessity of complementarity between detergents, as explained above. The following table describes precisely each

of the Phases 2 and 3 sub-stages required in order to assess and validate the selected alternative:

PHASE 2 (CARRIED ON EACH HYDRAGEL® TECHNIQUE, INDIVIDUALLY)			
STEP	**DESCRIPTION**	**FULL-TIME EQUIVALENT (FTE)**	**DELAY**
FEASIBILITY STUDY	• **Preliminary tests of use of each candidate at the current concentration of Triton™ X-100:** - Control of expected performances, in particular in terms of sensitivity, reproducibility and reading of the result - Search for the optimal concentration zone, allowing to achieve perfectly identical performances • **Feasibility of the candidates on the other functions of the kit and covered by Use-2, Use-3 and Use-4: performance tests at different concentrations** • **Study of the accelerated stability of each candidate for the different uses and selection of the candidate (s) selected for further studies**	3	9 months minimum
DRAFTING SPECIFICATIONS	• **Risk analysis (use, production)** • **Design constraints and functional requirements** • **Performance requirements** • **Regulatory requirements** • **Planning**		1 month

Table 17. Road map defined for Phase 2 dedicated to preliminary R&D steps

PHASE 3 & 4 (CARRIED ON EACH HYDRAGEL® TECHNIQUE, INDIVIDUALLY)			
STEP	**DESCRIPTION**	**FULL-TIME EQUIVALENT (FTE)**	**DELAY**
OPTIMIZATION (PHASE 3)	• **Optimization of the conditions of use of the selected candidate (concentration, electrophoresis parameters, etc.)** • Optimization of the use conditions of the selected candidate in the context of Use-1 (concentration, electrophoresis parameters, temperature, preparative phase, etc.) • Optimization of the use conditions of the selected candidate, in the context of Use-2 and 3, in particular: compatibility with the process of casting gels in order to obtain a better spreading of the agarose gel on the film, with washing solutions, dyes, colouring bases, with incubation conditions of the reagents on the gels, with antisera. • **Audit of analytical performances** • **Accelerated stability study of each kit subassembly containing the successful candidate** • **Development of HYDRASYS® migration / incubation / colouring programs and modification of the software if necessary** • **Development of a program of automatic reading if necessary, with possible modification of the software**	2	10 months minimum
MANUFACTURE OF TEST BATCHES (PHASE 4)	• **Production in production of a test batch (gels, strips, …)** • **Generation of HYDRASYS® and Phoresis® software versions** • **Accelerated stability study of each kit subassembly containing the selected candidate** • **Verification of the analytical performances and other requirements and comparison with the results obtained on batches manufactured in the laboratory during the optimization phase** • **Updated list of design constraints and functional requirements** • **Update of regulatory requirements** • **Modification and validation of the process of production of plastic supports**		4 months minimum

Table 18. Road map of Phases 3 and 4: R&D optimization and final analytical performances validation

→ *Phase 5 & 6 – Industrial validation of the substitution - External clinical testing and validation*

Again, the steps constituting phases 5 and 6 are already structured. In particular, the planning of the industrialization phase has been the subject of an intensive work, given the extent of the potential impacts that could occur in case of substitution and that it was necessary to anticipate. In the same way as before, all the sub-steps are listed in the table below, as well as the necessary resources.

PHASES 5 & 6 (CARRIED ON EACH HYDRAGEL® TECHNIQUE, INDIVIDUALLY)			
STEP	**DESCRIPTION**	**FULL-TIME EQUIVALENT (FTE)**	**DELAY**
MANUFACTURE OF PILOTS BATCHES	• **Writing a verification / validation protocol** • **Industrialization nomenclature** • **Review of risk analysis (use and manufacture)** • **Production of pilot batches (gels, strips and other subassemblies)**	2	2 months minimum
VERIFICATION/ VALIDATION	• **Performing tests according to the verification protocol** • **External validation (at a customer's) of the new kit containing the successful candidate** • **Real-time stability monitoring**		6 months (external validation time unknown and real-time stability time not counted
TRANSFERT	• **Provision of all necessary elements for the kits' regulatory compliance, for the kits production and placing on the market**		1 month

Table 19. Road map planned for Phases 5 and 6

More precisely, as explained above, the industrialization process groups eight processes for which a precise schedule is reported in the following table (***Table 20***). Indeed, workflow is divided in 5 phases: preliminary study, development, equipment qualification, process validation and summary. The stages impacted by the change are also specified.

So, with 534 weeks of work required for the industrialization of one kit involving the potential new detergent, the duration of industrialization would rise to about 10 years per kit.

Nevertheless, the durations mentioned here are related to "Project durations" and SEBIA does not have the resources to conduct all these projects in parallel. Only two people can today carry out the tests and realize the corresponding files. This means that only two *major equipment* (complex equipment) or four *minor equipment* (less complex equipment) can be made in parallel[58].

As a first approach, and without a real insurance of success, 18 major equipment and 9 minor equipment could be developed in parallel, **corresponding to**

[58] "Minor" or "major" equipment are defined according to a rapid risk analysis to determine the criticality of the passage for equipment from the use of Triton™ X-100 to a substitute.

approximately 9 years of continuous work carried out by 2 FTE in the context of this phase of industrialization.

PHASES 5 : INDUSTRIALIZATION DESCRIPTION (IN WEEKS)							
PROCESS	PRELIMINARY STUDY	DEVELOPMENT	EQUIPMENT QUALIFICATION	PROCESS VALIDATION	SUMMARY	TOTAL TIME REQUIRED FOR EACH PROCESS	IMPACT LEVEL
Semi-automatic distribution	2	4	6	-	2	14	Minor impact
Bottling (small volumes)	2	4	12	-	2	20	Minor impact
Strips manufacturing	14	28-40	48	32	8	142	Minor impact
Solutions preparation	16	32-48	48	32	8	152	Minor impact
Fluid system for transfer from storage ranks room to process lines	2	4	6	-	2	14	Major impact - Qualification time proportional to equipment complexity
Bottling (larger volumes – 100, 250, 700 mL)	10	20-24	36	24	10	104	Variable impact regarding the line and thus the equipment complexity
Software bars manufacturing	8	16-20	40	24	6	88	Variable impact regarding the line and thus the equipment complexity
Gel casting in clean rooms	20	40-60	72	40	20	202	Major impact regarding the equipment complexity

Table 20. Industrialization processes, impacts and timeline

→ *Phase 7 - Regulatory registration*

Once the validation data obtained by the R & D laboratories, the technical file and the CE marking file will be updated. This operation takes three months for a product range.

As briefly introduced, the constitution of the CE marking file makes it possible to make the declaration of CE marking and to put the products on the market in the European Union and in the European countries which recognize the CE marking (Switzerland, Norway, Iceland and Liechtenstein). In addition, the regulation of *in vitro* diagnostic medical devices varies with countries. For each country, the files are constructed from the data in the technical file according to their requirements. As a result, the time required for the regulatory procedure can dramatically vary from one country to another.

For example, China has very high requirements, and all validation studies must be done with several batches of reagents. In addition, studies in Chinese hospitals are needed.

As explained previously, in order to be able to sell products in the United States, SEBIA has to register them at the FDA. In case of an important change, a

correlation study done in the United States is necessary. The presentation of the results of the studies and the submission file is very framed.

Thus, according to the table in Appendix 1, the time required to register a single file in all of SEBIA's sales countries is approximately two years and two months. This time was calculated on the basis of the time required for registrations according to the regulations in force today. Nevertheless, it is likely that some regulations will increase their requirements in the future.

→ ***Phase 8 - Commercial deployment: system changes, customer management and validation***

This phase is essentially based on two steps, namely:

- Modification of documents relating to quality and safety, namely update of SDSs and translation in the 31 languages of the destination countries, possible modification of the packaging (label) in the event of modification of toxicity, update of the internal documentation and external. A minimum period of five months is required per product;
- Validation of the kit by the customer: Traditionally this step is carried out by the SEBIA application engineer. This consists of setting up and installing the software, installing the system on a network, merging the databases and setting up the hardware. Half a day is considered necessary for the accreditation of gel kits by a single client.[59]

4.2.1. Substitution timeline

According to elements presented in the previous sections, and considering unchanged work and process conditions, the global review period, summarized in the Table just below could be the following:

PHASE	TIME	RESOURCES
R&D	33 months / technique (considering an average of 3 kits/technique) = 122 years	3 FTE (to be recruited)
Industrialization	133 months (534 weeks)/kit =17,689 months for all kits	2 FTE (to be recruited)
Modification of quality & safety documents and packaging	5 months/product = 665 months	1 FTE (absorbable by the existing staff)
Regulatory compliance/Registration	74 years to register the 133 HYDRAGEL® kits concerned in all countries	1 FTE (to be recruited)
Customer validation and deployment	4 years according to SEBIA estimations 0.5 day/technique/customer (around 17 days/customer)	X FTE (to be recruited – regarding the customers' number)

Table 21. Substitution timeline in "current" conditions

[59] According to available data, it was not possible to define the precise number of customers.

Nevertheless, SEBIA, aware that a special effort will have to be provided, and this, in order to replace as quickly as possible, has built a shortened timeline of 12 years (***Table 22***):

	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033
Technical feasibility at R&D scale	●	●	●	●	●										
Optimization of industrial scale conditions and industrialization			●	●	●	●	●	●	●	●	●				
Regulatory registration								●	●	●	●	●	●		
Commercial deployment											●	●	●	●	●

Table 22. General substitution timeline for Use-1

The substitution duration can be further justified as follows[60]:

- A minimum **period of 5 years for R&D studies and technical feasibility and of 9 years for optimization of industrial scale conditions and industrialization** phases could be required. This calculation is based on[61]:
    - A preliminary estimation of a nine-year period of continuous work for the industrialization stage and related to an uncompressible and maximum number of eighteen major and minor nine equipment employed simultaneously, justifying a first R&D work carried out on 9 kits in parallel;
    - The recruitment of staff entirely dedicated to the phase of R&D and technical feasibility. It has been estimated that **27 FTE** will have to be recruited to carry out this work in five years, a time considered suitable to obtain satisfactory results, according to the uncertainty relative to the identification of efficient formulations and combined detergents formulations.
    - An industrialization phase that could thus start two to three years after the beginning of the R&D phase (allowing the industrialization processing of 9x2 = 18 kits by major equipment);
- A **regulatory compliance** that will have to be carried out in each country concerned, since SEBIA supplies all over the world, and requiring a procedural time of approximately 2 years for each kit. So, considering, in

[60] According to SEBIA internal calculations and estimations.
[61] Please refer to Annexes for calculation.

a best case, the simultaneous registration of several kits and the recruitment of additional staff, SEBIA has planned **a six-year duration** for all the products to be re-registered. In this context, **12 additional FTE** will have to be recruited;

- Due to the particular requirements of Sebia's customers and the variety of applications, a commercial deployment work will be undertaken and will require, for kit users, sustained and perfectly adapted support for change which could require a 5-year period, thus covering the offer of the 133 products concerned by Use-1;
- **Finally, this substitution timeline will also depend on results obtained in the context of other Uses of the current AfA, for which twelve-year and seven-year periods are required, according to the technical complexity of substituting 4-tert-OPnEO, number of products concerned and to be substituted and time needed for registration of corresponding new products.**

So, as presented, according to estimations based on resources dedicated to the development of one technique containing the alternative substance, the substitution could require the recruitment of many new R&D engineers and industrialization engineers and technicians (***Table 23***). Consequently, the replacement of Triton® X-100 could represent a significant cost for SEBIA. So, based on recruitment numbers estimations notified just above, and excluding additional staff employed for the customer validation phase[62] additional costs linked to the recruitment of staff dedicated to the substitution initiative could reach **€ 11.5M.**

FUNCTION	FTE TO BE RECRUITED	AVERAGE ANNUAL GROSS WAGE [63] (in €)	PERIOD OF ACTIVITY STRICTLY DEDICATED TO SUBSTITUTION PROJECT	COST LINKED TO NEW RECRUITMENT (in €)
R&D	27	58,000	4.5	7,071,167
INDUSTRIALIZATION	2	58,000	9	1,058,784
REGULATORY COMPLIANCE/REGISTRATION	12.4	45,000	6	3,341,625
TOTAL	**47.6**	-	-	**11,471,576**

Table 23. Additional FTE needed for the implementation of the substitution initiative and related costs

In conclusion, even if SEBIA will do its best to replace the substance in a potential time of 12 years, the financial costs related solely to the employment of staff is considerable and could economically impact the Group.

[62] No data about customers number available.

[63] Considering the recruitment of both technicians and engineers.

4.3. The most likely "non-use" scenario

In the context of Use-1, the relocation or sub-contracting of the production of HYDRAGEL® buffers outside the European Union cannot be considered, as their production:

- Has a strong human factor and requires an extremely high level of know-how;
- Is based on an unconditional commitment of shareholders of SEBIA to keep production in France. In addition, this provision is framed by the French Ministry of Finance;
- Follows a very stringent batch qualification process that requires an extremely low batch-to-batch variability and for which any deviation implies the destruction of batches.

A downgrade of product performances, that may arise from a non-optimal substitution, cannot be considered either, given the extremely high level of requirement in terms of reliability of diagnostics provided to end-users and of patient follow-up. So, in the context of a substitution, the new solution will be strictly validated in order to provide same test sensitivity, specificity and precision. In this context, the most likely "non-use" scenario is a permanent cessation of production of HYDRAGEL® kits. Strong impacts are foreseen in this scenario, notably:

- **Direct impacts** – loss of revenues and profits, loss of employment;
- **Indirect impacts** – impacts on patient health and national healthcare systems.

POTENTIAL "NON-USE" SCENARIO	HYDRAGEL® RANGES INVOLVING TRITON™ X-100
Downgrade of performances (substitution)	✗ This way is unacceptable for customers given the extremely high level of requirement for *in vitro* clinical diagnostics applications (potential impacts on human health in case of false result)
Relocation outside the EU	✗ The production is not transferrable considering that there is a contractual commitment of shareholders controlled by the French Ministry of Finance, to keep the production in France. Moreover, concerning REXOR, - The expertise and the equipment are concentrated in Paladru; - It's not possible to obtain from REXOR its relocation outside EU regarding some huge economic impacts.
Subcontracting outside the EU	✗ The loss of this strategic activity is not possible considering that there is a contractual commitment of shareholders controlled by the French Ministry of Finance, to keep the production in France. Moreover, the time relative to identify another subcontractor than REXOR located outside EU, and to contract with, which would need the manufacturing of adapted equipment, or a building dedicated to the new process line, could be critical. In addition, a subcontractor change could induce some financial penalties for SEBIA, as the result of breach of contract.
Cease of production	✓

Table 24. Potential "non-use" scenario

5. IMPACTS OF GRANTING AN AUTHORISATION

Collection and disposal of production wastewaters containing 4-tert-OPnEO is being contemplated by the sunset date of the substance.
In these conditions, the only realistic assumption for the assessment of costs per reduced emission is the "non-use" scenario, as all other emission avoidance initiatives have already been performed.

As explained previously, significant efforts have been already made by SEBIA towards the management of environmental risks associated to the use of 4-tert-OPnEO, through the use of an evapo-concentrator collecting a large part of releases containing 4-tert-OPnEO. Moreover, before the Sunset date, this system will be applied to the collection of remaining discharges of solutions containing 4-tert-OPnEO (in residual quantities).

In these conditions, any further reduction in emission can only be envisaged as a cessation of use of the substance; as demonstrated in section 4.5, such a situation corresponds to the "non-use" scenario for Use-1: a permanent cessation of the production of HYDRAGEL® kits and consequently a cessation of use of 4-tert-OPnEO.

The cost-effectiveness analysis will therefore be laid out as:

$$\frac{Economic\ costs\ of\ the\ "non-use"\ scenario\ (€)}{Planned\ substance\ release\ on\ one\ year(kg)}$$

An additional perspective will be provided in the form of a discussion regarding cost assessment of environmental impacts in the context of the present AfA.

5.1. Cost-effectiveness analysis

As explained in introduction, all uses constituting this dossier are interrelated. Indeed,

- **Use-1 concerns all gel electrophoresis kits**;
- **Uses-2 and 3 relate to functions complementary to those described in Use-1**. Thus, the absence of an alternative solution for one of the functions developed in Use-2 and / or Use-3 (ex: property of lysis - Use-3) will cancel any success of substitution obtained for a complementary function (ex: property of lipoprotein immobilization - Use-2). As a consequence, if one of the Uses did not benefit from the Authorisation of use of the substance, the other uses would be equally impacted, one of the functions of the detergent, then missing, preventing the good achievement of the test, rendered ineffective;
- The absence of an alternative solution on Use-4, which concerns only 3 products of the gel electrophoresis range, **has only influence on these 3**

products. On the other hand, again, if no alternative was identified on Uses-1, -2 & -3, the 3 products concerned by Use-4 could no longer be produced.

In this context, as previously stated, in case of a non-use scenario, the whole range of gel electrophoresis could no longer be produced. Thus, given the turnover of this range and the associated market share, it would no longer be possible for SEBIA to survive. In this context, the impact would be major, with the closure of all sites and its divisions and subsidiaries and the layoff of all employees of SEBIA. The scenario presented in Use-1 thus constitutes the reference scenario, uses being correlated with each other. In conclusion, whatever the use, the real socio-economic scenario is therefore that presented in this Use-1 file, concerning all HYDRAGEL® products.

5.2. Economic impacts

The ceasing of production and commercialisation of HYDRAGEL® kits would entail both direct impacts (loss of revenues directly related to those products) as well as indirect impacts (loss of revenues and profits related to markets that would be lost to SEBIA in case of unavailability of HYDRAGEL® kits in SEBIA's portfolio).

In the special case of REXOR, the consequences of a cessation of production of SEBIA would not have a critical impact on the subcontracting Company survival. Indeed, REXOR is not in a situation of economic dependence, but a stoppage of SEBIA's orders could at least result in a reduction in revenue, and at most imply a restructuring of the subcontractor. For this reason, the socio-economic analysis has only been done for SEBIA. So, REXOR is excluded from this scope.

5.2.1. Loss of revenues

The average of revenues associated with products of Use-1 for the years 2015, 2016 and 2017 are synthesised in what follows. Revenues associated with HYDRAGEL® range products are as follows:

YEARS	REVENUES (in €)
2015	75,577,989
2016	82,042,764
2017	85,479,210
AVERAGE	**81,033,321**

Table 25. Revenues associated with HYDRAGEL® products concerned by Use-1

The electrophoresis offer concerned by Use-1 represents around 35 % of the global turnover of SEBIA. A total of an **average € 81,033,321 annual revenue** is related to the commercialisation of products of Use-1.

So, the total revenues loss over the 2021-2033 period, is reported in the following table. The calculation is based on the duration of impact linked to loss of revenues for HYDRAGEL® products range as presented in the definition of the "non-use" scenario (equal or more than ten years), as well as an average 8% annual profits growth on which is based the Company financial viability.

Indeed, the SEBIA group belongs to investment funds, it is a so-called LBO structure in which the group is heavily indebted (up to a billion euros) and must therefore generate surplus cash to pay interest to debt and proceed to its repayment. The group is required to test its financial strength on a quarterly basis. In the presence of a sharp slowdown in activity SEBIA would be unable to settle its debt maturities and the group would quickly fall into receivership. The cash surpluses to be generated are estimated at 8% per year, which SEBIA has realized for several years, justifying this annual growth of 8%.

Nominal value of revenues loss	**€ 2,194,210,789**
Discounted value of revenues loss	**€ 1,494,314,773**

Table 26. Total revenues loss over the impact period for Use-1
(*): considering a 4% discount rate

With a loss of revenues of around € 1.5B over the 2021-2033 period, the "non-use" scenario will generate highly critical impacts on the economic activity of SEBIA and will certainly jeopardise its very survival, resulting in the bankruptcy of the Company.

5.2.2. Loss of markets

HYDRAGEL® range is not only a major source of revenues for SEBIA, but it also constitutes key elements of SEBIA's portfolio insofar as they are a prerequisite for accessing calls for tenders.

Half of SEBIA's customers are public customers and consequently, practice regular calls for tenders (periodicity of three to five years depending on markets maturity) issued by countries, and covering several diagnostic solutions. Electrophoresis constitutes a major technique in the detection of various pathogens and diseases. It is mandatory for any potential participant in the tendering process to possess a detection and follow-up solution based on proteins identification in its portfolio. Moreover, these tenders are often specific to SEBIA's specialties. Consequently, the loss of HYDRAGEL® kits would have a significant knock-off effect on sales of these products but also on sales of other ranges of SEBIA's portfolio. Several markets would thereby be closed to SEBIA for a significant period of time.

A proper quantification of such a market loss can hardly be carried out. The following elements, however, can be brought forward to characterise the knock-off effect of the "non-use" scenario on sales of other products of SEBIA's portfolio:

- Tenders are issued for a duration of three to ten years depending on countries. Should a tender be closed to SEBIA due to missing critical

products in its portfolio, then said market would be lost for such a long period of time, thereby jeopardising revenues for SEBIA over a medium to long duration.

- Tenders represent a major part of SEBIA's revenues: today, the turnover share realized through these calls for tenders are estimated by SEBIA at 50%. Consequently, their loss will significantly impact the financial position of the Company.

5.2.3. Penalties linked to the range discontinuation

Due to contracts between SEBIA and its customers, particularly in the case of exclusive supply of certain products, the HYDRAGEL® range discontinuation could lead to a sudden break in certain commercial commitments. In the event of breach of contract, SEBIA could then be exposed to lawsuits for non-compliance with contractual obligations and the payment of penalties. In the case of very specific tests such as those dedicated to the detection of Myeloma, the impacts of such a cessation would be threefold:

- Sanitary with deleterious consequences for the entire health chain due to the unavailability of these tests;
- Economic for SEBIA, related to the loss of direct income and to these financial penalties;
- In terms of reputation for SEBIA which today has a very positive image on the market.

In the current situation, it is extremely complex to quantify the amount of these penalties on the references concerned: it is indeed the whole of the customer-distributor relationship and therefore distributor-SEBIA that would cease. SEBIA would then be exposed to penalties corresponding to the losses realized by the distributor over the remaining duration of the contracts that was concluded with customers. In addition, these could be important since, to date, SEBIA products are marketed by specialized distributors in countries where SEBIA is not directly operating and can represent more than 25% of the activity of these distributors.

5.2.4. Discussion: net impact for the global chain

5.2.4.1. Discussion: net impact for the healthcare system

SEBIA is a pioneer and a leader in the diagnosis of numerous diseases based on blood abnormalities.

In these conditions, considering HYDRAGEL® ranges, and within the current knowledge, it is difficult to predict how would the sector adapt if SEBIA ceased to market the products of Use-1 and what the overall net impact for the Society of the "non-use" scenario would be. In addition, SEBIA has no knowledge regarding whether or not competitors use 4-tert-OPnEO for the production of their product and, if they do, whether or not they will be applying for an Authorisation.

Nevertheless, it can be argued that revalidating the substitute buffers or switching to a competitor system would generate substantial economic impacts on healthcare systems, especially, given national healthcare systems limited budgets, if the range discontinuation or the Triton™ X-100 substitution happened in the middle of the contract and lifecycle of the diagnostic solution. Consequently, it could then result in negative effects on both the healthcare system and the patients themselves.

Moreover, SEBIA has a highly sought-after expertise, particularly in the field of myeloma screening for which the Company has exclusive rights. In order to stand out, SEBIA has also chosen to move towards the diagnosis of rare diseases such as undetermined monoclonal gammopathies, Waldenström diseases and the development of extremely specific tests (A1AT ISO and β2-transferrin). With such a monopoly, and in the absence of competing solutions, a discontinuation of these special ranges would lead to a sudden break in the global management of some rare diseases.

5.2.4.2. Discussion: net impact for the supply chain

SEBIA makes 80% of its purchases from 60 different suppliers. The most important of these are dedicated to the supply of packaging and subcontracting in electronic equipment: these are intended for at least one production line for SEBIA products. Consequently, a suspension of orders from SEBIA would necessarily entail a restructuring of these suppliers, or even a cessation of activity (only for one of them which is in a situation of economic dependence).

In addition, as explained in the previous section, the marketing of SEBIA products is carried out by specialized distributors in countries where SEBIA does not have a direct presence: the sale of SEBIA products can thus represent, as explained in the previous section, more than 25% of the activity of these distributors. A stop of the marketing would imply there again a restructuring at these distributors.

The net impact for the Society of SEBIA's "non-use" scenario cannot be estimated in a reliable way. It can however be stated that no competitor will be able to supplant SEBIA for the electrophoresis products of Use-1 on a short to medium term basis. Indeed, given [1] the large market share of SEBIA in the electrophoresis assays sector, [2] the very high level of know-how required, [3] the monopoly of SEBIA on certain tests concerning the diagnosis of rare diseases, [4] the extremely capital intensive nature of the production of IVD products and [5] the lack of capacity of SEBIA's competitors in the supply of associated equipment, it is very unlikely that competitors will be able to ramp up quickly their production of alternative solutions. Consequently, this situation could be critical in economic but also in sanitary terms for the medical sector, especially for the diagnosis of rare diseases (Myeloma, von Willebrand and Waldenström diseases, …) for which SEBIA has exclusivity with certain laboratories.

Moreover, the entire value chain could be indirectly impacted by a potential cease of production : more particularly, packaging and electronics suppliers but also IVD devices distributors could have to engage a restructuration of their activity.

5.2.5. General conclusion on economic impacts

The HYDRAGEL® range is extremely strategic for SEBIA, as [1] it represents a revenue of € 81M per year, which is a significant share of the Company's revenues, [2] they concern clinical products and thus a large applications panel, [3] are a source of significant revenues for SEBIA products distributors in countries where SEBIA is not directly established, and [4] a prerequisite to participate in call for tenders for other products.

5.3. Human health impact

Impacts on human health of the "non-use" scenario are detailed in the following section.

5.4. Social impact

Social impacts can be expected in the context of the "non-use" scenario, that fall into three categories:

- Medical impacts on patients that may face a disruption in diagnosis;
- Loss of employment that would arise from the cease of production.

5.4.1. Medical impacts

Products of SEBIA, and among them, products concerned by the present application for Authorisation, therefore provide a significant support in the diagnosis, the follow-up or the exclusion of numerous pathologies, on a global worldwide scale.

In what follows, it will be explored, both qualitatively and quantitively where possible, the medical stakes associated with products concerned by Use-1 of the present application of Authorisation.

5.4.1.1. Context

Context elements can be articulated as follows to give insight on the medical – social situation of Use-1 products.

→ *[1] SEBIA's electrophoresis assays under Use-1 is used as an efficient preparative tool involved in the diagnosis of a broad array of complex pathologies*

HYDRAGEL® assays concerns a vast array of pathologies essentially based on blood proteins anomalies:

- Multiple Myeloma, a rare bone marrow cancer, Waldenströme disease and other undetermined monoclonal gammopathies;
- Haemoglobin abnormalities generating pathologies like thalassemia;

- Chronic diseases characterized by:
  - Enzymatic dysfunction (Case of serious liver diseases)
  - Enzymatic overproduction frequently occurring in the context of myocardial infarction
  - A modified protein synthesis (for example, presence of β2-transferrine revealing a break of the meningeal barrier)
- Other mental illnesses like alcoholism, anorexia, …

Consequently, HYDRAGEL® kits can be used in different medical specialties like internal medicine, paediatrics, cardiology, oncology, gastro-enterology, …

The disruption of supply of SEBIA's Use-1 HYDRAGEL® solution would therefore impact diagnosis of a broad scope of rare diseases and difficult to diagnose.

→ *[2] They are integrated in a global diagnosis system which supports efficiently a valuable decision*

Products of Use-1 allows to diagnose multiple haemoglobin and other proteins abnormalities which will be detected and if necessary, quantified. This diagnostic tool is a crucial step and thus very helpful in the establishment of medical diagnostics or the follow-up of treatments.

→ *[3] Several obstacles will complexify the adoption of alternative solutions by the current users of SEBIA solutions*

HYDRAGEL® kits have been specifically developed to be exclusively used on SEBIA instruments. Conversely, it is not possible to use other kits with SEBIA equipment.

Alternatively, there is the possibility for hospitals, laboratories and other customers to move to equipment and products of a competing company that is not subject to Authorisation under REACh. In such a case, these customers would have to purchase new equipment (instrument, computer) and train personnel. In this scenario, the availability of such equipment is very low as SEBIA represents around 95 % of the overall European electrophoresis market. With 16,000 equipment units installed worldwide, it is therefore unlikely that one or several actors will be able to ramp up their production to supply such a high volume of instruments and kits.

There are, however, several impediments to such a change in supplier for IVD services, notably:

- Hospitals and laboratories usually have global contracts to optimise costs. As they use the SEBIA's electrophoresis kits, the change of supplier will therefore generate either over-costs or the need to reissue an invitation to tender for these tests (which may not possible if the alternative supplier does not offer a comparable test portfolio or allow or ad hoc use of their system);
- Operators will have to be trained to use materials from a competitor;

- The test methods offered by competitors may not provide the same level of performance as that of SEBIA system.

→ *[4] In conclusion, the unavailability of SEBIA's Use-1 products will generate impacts on patients, hospitals and healthcare systems*

The disruption of supply of SEBIA's buffers included in Use-1 assays to the medical sector in the context of the "non-use" scenario would have an impact on hospitals and laboratories that will ultimately be passed to patients. The extent of that impact can hardly be characterised as it would essentially require to model the reconfiguration of the entire IVD sector in the EU.

So, on the basis of the elements brought forward in the previous sections, it can however be stated that the "non-use" scenario would have significant economic impacts for the operators (hospitals, laboratories) that will have to purchase new diagnostic equipment and would generate a significant need for adaptation of the medical sector (personnel training for new methods and equipment). In addition, there will be associated costs effort and elapsed time to revalidate their menus, and in some jurisdictions, the resubmission / registration with their regulatory bodies.

5.4.1.2. Complementary elements

According to the various pathologies diagnosed by the HYDRAGEL® kits and to the global impacts of these diseases, this electrophoresis solution is part of an essential diagnosis tool, in particular in the earlier detection of the pathologies, resulting in the mortality decrease and in the reduction of costs relative to treatment, hospitalization, work stoppages, …

As examples, the main data collected for some of major diseases diagnosed through HYDRAGEL® techniques are the following:

→ *Multiple Myeloma*

- Multiple myeloma or Kahler Disease is really rare: in France, the incidence is 4,000 cases per year. In the United States, 45,000 people live with myeloma with approximately 20,000 new cases a year.[64] Its incidence tends to increase.
- The median survival is 62 months for phase 1 of the disease, 45 months for phase 2 of the disease, and 29 months for phase 3 of the disease, justifying the urgency of detecting the disease as quickly as possible [65].
- Myeloma is the second most common haematological disorder (10%) after non-Hodgkin's lymphoma. It accounts for about 1% of all cancers and 2% of all cancer deaths.

[64] Raab M. S. *and coll.* Multiple Myeloma. *Lancet* ***2009***, 374, 324-339.

[65] *Survie médiane* - http://jco.ascopubs.org/cgi/content-nw/full/23/15/3412/F1

→ *Thalassemia*

- The global prevalence of β-thalassemia is estimated at 288 000 cases worldwide, 60 to 80% of which require treatment[66] The annual incidence of symptomatic cases would be of the order of 1 per thousand inhabitants in the world and 1 in 10,000 in Europe[67].
- As it is a genetic disease, and due to migration and mixing, the gene is found in all parts of the world, in a 1.5% of the world population (80 to 90 million people).
- In the United States and the European Union, the prevalence is estimated at 15 000 cases, with 1500 children born each year with the disease. In most other regions (North Africa, Middle East, Asia), access to treatment is limited and patients are at increased risk from an early age.
- In the case of thalassemia major, without treatment, the life expectancy of the child hardly exceeds 20 years.

→ *Myocardial Infarction[68]*

- Each year cardiovascular disease (CVD) causes 3.9 million deaths in Europe and over 1.8 million deaths in the European Union (EU).
- CVD and thus myocardial infarction accounts for 45% of all deaths in Europe and 37% of all deaths in the EU.
- CVD is the main cause of death in men in all but 12 countries of Europe and is the main cause of death in women in all but two countries.
- Death rates from both ischaemic heart disease (IHD) and stroke are generally higher in Central and Eastern Europe than in Northern, Southern and Western Europe.
- In 2015, there were just under 11.3 million new cases of CVD in Europe and 6.1 million new cases of CVD in the EU.
- In 2015, more than 85 million people in Europe were living with CVD and almost 49 million people were living with CVD in the EU.
- Over the past 25 years, the absolute number of CVD cases has increased in Europe and in the EU, with increases in the number of new CVD cases found in most countries.

→ *Severe hepatic insufficiency*

- The annual incidence in the United States is about 5 cases per one million inhabitants [69].
- The pathology is accompanied by significant morbidity and mortality, close to 40%.

[66] Biffi A. Gene Therapy as a Curative Option for beta-Thalassemia. *The New England Journal of Medicine* ***2018***, 378 (16), 1551-1552.

[67] Galanello R., Origa R. Beta-Thalassemi. *Orphanet Journal of Rare Diseases* ***2010***, 5,11.

[68] http://www.ehnheart.org/cvd-statistics.html

[69] Bower W.A. *and coll.* Population-based surveillance for acute liver failure. *Am. J. Gastroenterol.* ***2007***, 102, 2459-2463.

- Liver transplantation is sometimes the only option and allows a survival rate greater than 60% at 10 years [70].

5.4.1.3. Conclusion

So, according to these statistics, the role of diagnosis tools involved in the detection of rare and chronic pathologies is crucial, both in health safety and economic terms. Indeed, in the case of rare diseases like Waldenström disease or Multiple Myeloma, they are so infrequent that it is often difficult to diagnose as soon as the first symptoms appear. The patient is then in a diagnostic wandering which results in a multiplication of examinations and therapeutic protocols logically without results. In some cases, the absence or the delay of diagnosis can lead to an aggravation of the disease with significant consequences for the health of the patient and its management. Conversely, in the case of chronic diseases or pathologies or high incidence (cardiovascular diseases, acute liver failures, …), the number of patients which could have to be submitted to corresponding IVD tests, is tremendous.

Nevertheless, these values are subject to significant uncertainties. Moreover, as the distribution of used IVD assays is difficult to assess between the different technologies (immunoassays, microbiology technologies, …), it is not possible to evaluate the number of patients dependent on IVD immunoassays based on a first protein identification and quantification.

An evaluation of the number of patients which could be impacted by the unavailability of the HYDRAGEL® range, is possible, based on the number of kits sold by SEBIA and the number of tests carried out with these kits. Indeed, an average of **336,737 kits** concerned by Use-1 were sold per year between 2015 and 2017. According to data obtained from package inserts, and to numbers of samples tested per kit, **84,211,400 patients' samples**[71] could have been tested with kits concerned by Use-1. So, even though the exact chain of consequences of their unavailability can hardly be characterised in a robust manner, the unavailability of this electrophoresis solution would impact a very large number of patients on a worldwide scale.

In conclusion, the unavailability of the HYDRAGEL® reagents kits in the context of the "non-use" scenario will impact a significant number of patients.

5.4.2. Impact on employment

5.4.2.1. Number of jobs concerned

Usually, in this part, only actual working hours of workers dedicated to activities associated to products concerned by AfA are considered as potentially lost in the context of the "non-use" scenario.

[70] Germani G., Theocharidou E., Adam R. *et al.* Liver transplantation for acute liver failure in Europe: outcomes over 20 years from the ELTR database. *J. Hepatol.* ***2012***, 57, 288-296.

[71] Please refer to Appendix 1.

Nevertheless, in this case, and as precised previously, all the HYDRAGEL® ranges are concerned. In this context, with a loss of income rising around 40 %, and in the case of the cease of the production of these kits, the Company would not be able to continue its activities. Moreover, actually, SEBIA sites worldwide would be concerned. Consequently, the economic impact would be so deleterious that the closure of SEBIA and its subsidiaries would be inevitable.

So, in order to place the assessment of the impacts on employment on assumptions considered realistic (according to SEBIA), all of the jobs of SEBIA and its subsidiaries would be considered as potentially lost in the context of the "non-use" scenario.

So, even if 133 jobs were identified as directly and exclusively dedicated to Use-1 products activities, considering the closure of the Company, **around 550 jobs** could be affected by the cease of production of the HYDRAGEL® range under Use-1.

5.4.2.2. Assessment via default value

SEAC's note[72] proposes a default welfare cost factor value of 2.7 for the assessment of costs associated with unemployment.

Following this method, the social value of jobs lost in the context of the "non-use" scenario for Use-1 can be estimated as follows:

Welfare cost factor	2.7
Gross wages of workers	€ 68,500[73]
Number of jobs lost	550
Social value of jobs lost	**€ 101,722,500**

Table 27. Calculation of the social value of jobs lost via the default value methodology for Use-1

5.4.2.3. Detailed assessment

A complementary assessment is performed in what follows in view of providing a more specific characterisation of the cost related to unemployment in the context of Use-1. This assessment is largely based on the framework drafted by R. Dubourg[74].

The following impact categories will be explored:

- Value of output/wages lost during the period of unemployment
- Impact of being made unemployed on future earnings and employment possibilities ('scarring' effect)
- Cost of searching for a new job

[72] ECHA, SEAC/32/2016/04 - 32nd meeting of the committee for socio-economic analysis, 6-15 September 2016, Helsinki, Finland

[73] Calculation based on employees categories and status, regulations applied in the countries concerned anf their change rates (Source : SEBIA).

[74] R. Dubourg, Valuing the social costs of job losses in applications for autorisation, The Economics Interface Limited, ***September 2016***.

- Recruitment costs
- Leisure time

For each impact categories, it was attempted to rely as much as possible on data specific to the situation of SEBIA' sites in France. Generic data, however, have had to be used where specific data was unavailable.

→ *Value of output/wages lost during the period of unemployment*

The value of output (wages) lost during the period of unemployment was calculated using the gross wages of workers concerned and the average duration of unemployment in France (388 days[75]):

Gross wages of workers	€ 68,500
Average duration of unemployment in France	388 days
Nominal value of lost output due to the initial unemployment spell	**€ 72,816**
Discounted value of lost output due to the initial unemployment spell	**€ 62,102**

Table 28. Calculation of the value of output/wages lost during the period of unemployment for one job, Use-1

→ *Impact of being made unemployed on future earnings and employment possibilities ('scarring' effect)*

Scarring effect reflects the tendency to obtain a job with lower wages when unemployed compared to when employed. A scarring effect value of 20% is proposed by Dubourg and will be used in the present assessment.

Scarring effect (average reduction in output following reemployment)	20%
Duration of scarring effect	5 years
Nominal value of lost output due to scarring	**€ 68,500**
Discounted value of lost output due to scarring	**€ 48,202**

Table 29. Calculation of the value of Discounted value of lost output due to scarring for one job, Use-1

→ *Cost of searching for a new job*

The value for unemployed persons of time spent searching for a new job can be roughly estimated via data proposed by Dubourg (it is considered an average of 2.5 hours spent per week searching for a job) and hourly wages derived from the expected "scarred" gross wages. Please note that the figure of time spent searching for a new job is a rough estimate whose main purpose is to provide an order of magnitude of this cost item.

[75] Pôle Emploi, la durée du chômage de stabilise au 2ème trimestre 2018, Indicators n°18.034.

Weekly time spend looking for a new job	2.5 hours
Hourly wages (*)	€ 33,3
Duration of unemployment	55 weeks
Nominal value of time lost searching for a new job	**€ 4,616**
Discounted value of time lost searching for a new job	**€ 3,937**

Table 30. Calculation of the value of output/wages lost during the period of unemployment for one job, Use-1

(*) Considering 35 hours per week and 5 weeks of vacation per year

→ *Recruitment costs*

The assessment of recruitment costs (cost of hiring employees) is carried out considering a global of 30% of expected annual gross wages for the unemployed person. Please note that, as per the previous impact category, the figure for recruitment cost is a rough estimate whose main purpose is to provide an order of magnitude of this cost item.

Recruitment costs (percentage of expected annual gross wages)	30%
Gross ('scarred') wages	€ 68,500
Nominal value of recruitment costs	**€ 20,550**
Discounted value of recruitment costs	**€ 16,851**

Table 31. Calculation of the recruitment costs for one job, Use-1

→ *Leisure time*

The assessment of leisure time aims at characterising the value of time freed from work due to unemployment. As per Dubourg, a reservation wage[76] of 80% of expected post-tax wages is considered.

Reservation wages (of expected post-tax wage)	80%
Expected 'scarred' gross wages	€ 54,800
Average personal tax on wages	30%
Average duration of unemployment (days)	388
Nominal value of benefits from leisure time	**€ 36,222**
Discounted value of benefits from leisure time	**€ 27,822**

Table 32. Calculation of the value of benefits from leisure time related to unemployment for one job, Use-1

[76] From Dubourg: "The reservation wage is the point at which the individual is just indifferent between working and not working"

→ *Total cost of unemployment*

Total costs of the loss of one job on the review period and on one year are detailed in what follows:

	COSTS OF THE LOSS OF ONE JOB ON THE REVIEW PERIOD	COSTS OF THE LOSS OF ONE JOB ON ONE YEAR
Lost output	€ 62,102	€ 58,554
Job search	€ 3,937	€ 3,712
Recruitment costs	€ 16,851	€ 16,851
Scarring	€ 50,129	€ 10,827
Leisure time	- € 27,822	- € 26,232
TOTAL	€ 105,198	€ 63,712

Table 33. Costs of unemployment on the review period and on one year, discounted

Considering the number of jobs lost foreseen in the context of the "non-use" scenario for Use-4 (550 jobs), the total cost of unemployment amounts to:

- **€ 57,858,642 on the entire review period;**
- **€ 35,041,729 on one year.**

5.4.2.4. Comparison of assessment via default value and detailed assessment

Assessment via default value	€ 101,722,500
Detailed assessment	€ 57,858,642
Detailed assessment on yearly basis*	€ 35,041,729

Table 34. Comparison of the characterisation of costs of unemployment using the default value and the detail assessment methodologies *(* 2022 as the referent year)*

In a conservatory approach, the detailed assessment value will be used in the calculation of the monetised impacts of the "non-use" scenario.

5.4.3. Impacts on the global health chain

As discussed in the previous sections, products of Use-1 are widely used on a world scale by a vast array of medical specialties. In this context, wider economic impacts can be expected for several actors of the health chain. It will be shown in what follows that, from a global point of view, negative impacts associated with the

unavailability of diagnostics will outweigh potential positive impacts related to the potential market adaptation in favour of competitors.

5.4.3.1. Negative impacts

→ *A major disruption of the associated end-user sectors*

Multiple end-users rely, to various extends, on tests concerned by the present application. Their unavailability on the market will generate a major disruption in their functioning as replacements will have to be sourced, purchased, installed, operators trained to their use, followed by the instrument requalification and the revalidation of its assay menu.

→ *The competitors' capacity to produce and supply alternative tests is unknown*

Given [1] the biological and sensitive character of *in vitro* diagnostics products and [2] the large market share of SEBIA in electrophoresis-based assays, it appears unlikely that competitors will be able to provide alternative solutions in sufficient quantities on a short to medium term.

→ *Products of Use-1 are specifically designed to be used with SEBIA's machines*

HYDRAGEL® reagents kits are to be specifically used on SEBIA's automatons. Competing products cannot be directly used on this equipment. As a consequence, hospitals and laboratories that are equipped with SEBIA's equipment will not only have to source alternative testing solutions but also to purchase and calibrate new automated analysers as well as to train personnel to their use followed by the instrument requalification and the revalidation of its assay menu.

Major economic impacts will therefore be generated by the unavailability of products of Use-1 to the HYDRAGEL® range end-users.

5.4.3.2. Positive wider economic impacts

SEBIA's "non-use" scenario will generate an opportunity for competitors. The scale of these benefits cannot, however, be precisely assessed as:

- Medical diagnostics for pathologies addressed by products of Use-1 may be supported via a vast array of tests and methods depending on medical situations, practitioners' judgement and equipment available within each country medical structure (reimbursement and differential diagnosis guidelines) and patient demographic. It is therefore impossible to model the market adaptation to SEBIA's cease of supply of products of Use-1.
- Competitor's manufacturing capacities are unknown. In addition, it cannot be anticipated whether or not competitors will be able to supply solutions on a short-term basis after SEBIA's potential disruption of supply.

Nevertheless, it seems unrealistic for SEBIA competitors to offer an alternative solution in sufficient quantities.

- It is not known whether or not competitors use 4-tert-OPnEO to synthesise their products and if so, whether or not they will be granted an Authorisation for their use.

Considering these elements, it appears realistic to consider that SEBIA's "non-use" scenario will benefit to competitors. The extent of those benefits cannot, however, be modelled both in terms of identification of potential companies affected, their location (within or outside the EU) and the share of revenues lost by SEBIA that they will be able to recover.

5.5. Conclusion of the socio-economic analysis

5.5.1. Synthesis of the impacts of the "non-use" scenario

Main foreseeable quantitative impacts of the "non-use" scenario are synthesised in what follows:

CATEGORY	MONETISED IMPACT	MONETISED IMPACT ON YEARLY BASIS (2022)
Loss of revenues	**€ 1,494,314,773**	**€ 94,237,836**
Impact on employment	**€ 57,858,642**	**€ 35,041,729**
TOTAL	**€ 1,552,173,415**	**€ 129,279,565**

Table 35. Synthesis of monetised impacts of the "non-use" scenario, Use-1

To these monetised impacts can be added complementary impacts of major importance that can only be described in a qualitative manner:

CATEGORY	IMPACT
Loss of markets	A significant knock-on effect can be expected with the unavailability of products of Use-1 in SEBIA's portfolio
Medical impacts	A significant disruption and reorganisation of medical analysis activities on a worldwide scale would be generated in case of SEBIA's inability to supply products of Use-1
Global social impacts	Strong impacts on the health system of European and non-European countries and on the sanitary control of consumer products

Table 36. Synthesis of qualitative impacts of the "non-use" scenario, Use-1

5.5.2. Complementary element: cost-effectiveness ratio

As detailed in the methodological section, the cost-effectiveness ratio constitutes complementary characterisation metric of the socio-economic analysis.

To define it on the same time scale, this ratio will be done on an annual basis. 2022 is the reference year for this calculation.

In the context of SEBIA's industrial situation, releases of 4-tert-OPnEO are reduced to the minimum: to date, the only remaining release sources are residues in washed glassware and the potential releases of the substance are treated in the municipal waste water treatment system. So, today, the ratio calculation one year (2022) is the following:

$$Releases\ avoid\ \ C/E\ \ ratio = \frac{\mathbf{129,279,565}\ €}{\mathbf{0.03}\ (\boldsymbol{kg})} = \mathbf{4.31}\ \boldsymbol{x}\ \mathbf{10^9}\ € — \boldsymbol{kg}$$

Nevertheless, taking into account that:

- SEBIA will continue to implement all the measures required to limit releases into the sewers of substances that can lead to water pollution;
- The non-contamination process based on the use of the evapo-concentrator will be optimized and potentially enlarged to all activities, including those resulting in the current releases of 4-tert-OPnEO residues into the sewers;
- Quantities of 4-tert-OPnEO released will therefore remain less or equal to those rejected in 2018;

a release cost-effectiveness ratio would tend toward infinite, making a strong case for the benefits of continued use outweighing the risk.

5.6. Uncertainty analysis for both the "applied for use" and the "non-use" scenario: consumption cost-effectiveness ratio

A complementary perspective can be provided with consumption cost-effectiveness, as follows (solely taking into account the direct economic impacts for SEBIA of the cease of production of the SEBIA® reagents in the context of the "non-use" scenario). This calculation is based on the average annual consumption of SEBIA (See section 3.2.1).

$$Consumption\ \ C/E\ \ ratio = \frac{Economic\ costs\ of\ the\ "non-use"\ scenario\ (€)}{Substance\ consumption\ on\ the\ review\ period(kg)}$$

$$= \frac{\mathbf{1,552,173,415}\ €}{\mathbf{12\ x\ 62.19}\ (\boldsymbol{kg})} = \mathbf{2.08}\ \boldsymbol{x}\ \mathbf{10^6}\ € — \boldsymbol{kg}$$

5.7. General conclusion on the impacts of granting an authorisation

Even though no direct conclusion can be drawn on the basis of threshold values for releases cost-effectiveness ratio, it provides a complementary argument in favour to conclude that benefits of continued use outweigh widely the risk.

Considering this ratio, we can see that avoiding releases of less than one kilogram of 4-tert-OPnEO involves an unacceptable and disproportionate cost for SEBIA's business: as demonstrated previously, this could lead to the bankruptcy of the Company and the final economic costs could be much higher than those calculated previously.

A synthesis of the monetised impacts of the "non-use" scenario is provided below:

		MONETISED IMPACTS
Economic impacts	**Loss of revenues**	**€ 1,494,314,773**
Social impacts	**Loss of employment**	**€ 57,858,642**
Total monetised impacts of the "non-use" scenario		**€ 1,552,173,415**

Table 37. Synthesis of the monetised impacts of the "non-use" scenario

As a complement, other impacts of the "non-use" scenario are synthesised in the table below:

		IMPACTS	ORDER OF MAGNITUDE
Economic impacts	**Loss of markets**	A significant knock-off effect can be expected with the unavailability of products of Use-1 in SEBIA's portfolio	Hundreds of millions to ten of billions of Euros
Human health impact	**Impacts on human health**	From a global point of view, the "non-use" does not involve an overall reduction of risks for workers.	-
Wider impacts	**Impact on the society health system**	The "non-use" scenario will generate strong impacts on the health system of European and non-European countries and on the sanitary control of consumer products	Millions of persons impacted

Table 38. Other impacts of the "non-use" scenario

6. CONCLUSION

6.1. Comparison of the benefits and risks

No benefits/risks assessment can be performed for this substance. Nevertheless, the different documents show that ***the level of exposure is extremely low compare to:***

- ***the global society health system (millions of patients impacted worldwide);***
- ***the benefits for SEBIA company, which were estimated at around € 1.5B but could be much higher, considering the continuation of SEBIA activities.***

6.2. AoA-SEA in a nutshell

As explained in section 5.1., all uses constituting this dossier are interrelated. Indeed, **under Uses presented in this AfA are concerned different properties and functions offered by the detergent and required at different steps and levels of HYDRAGEL® assays, all included in the scope of the dossier.**

In this context, as previously stated, in case of a non-use scenario, the whole range of gel electrophoresis could no longer be produced. Thus, given the turnover of this range and the associated market share, it would no longer be possible for SEBIA to survive. In this context, the impact would be major, with the closure of all sites and its divisions and subsidiaries and the layoff of all employees of SEBIA. The scenario presented in Use-1 thus constitutes the reference scenario, uses being correlated with each other. In conclusion, whatever the use, the real socio-economic scenario is therefore that presented in the Use-1 file, concerning all HYDRAGEL® products.

AoA – SEA IN A NUTSHELL

APPLICATION FOR AUTHORISATION

APPLICANT: SEBIA

SUBSTANCE: 4-(1,1,3,3-Tetramethylbutyl)phenol, ethoxylated

USE: **Use-1**

Industrial use of 4-tert-OPnEO for its "wetting" properties allowing the dissolution, the dilution and the good spreading of substrates and reagents, necessary to optimize the sensitivity of the *in vitro* diagnostic test

ANALYSIS OF ALTERNATIVES

The main problematics for SEBIA regarding this type of products are the maintenance of the analytical and clinical performances and consequently, all the validation processes carried out both internally and externally, including that performed by the clients and the compulsory regulatory compliance in the event of a change of at least one device component or reagent formulation.

In this context, a preliminary internal work of research is currently carried out under easier process conditions and first results allow to consider one potential alternative to 4-(1,1,3,3-Tetramethylbutyl)phenol, ethoxylated : Tween® 20. However, considering that all analytical and clinical test performances are mainly linked to the ability of the substance to offer a good solubilisation and distribution of proteins of biological interest along the electrophoresis gel, and according to the high level of technical difficulties in keeping similar performances than those obtained with Triton® X-100, it will probably not be possible to confirm that this candidate is at least as reliable and efficient.

Consequently, as for now, no truly suitable alternative has been yet identified and is able to be developed, industrialised and qualified before the sunset date of 4-(1,1,3,3-Tetramethylbutyl)phenol, ethoxylated and a **twelve-year review period** is needed to achieve substitution.

SOCIO-ECONOMIC ANALYSIS

No benefits/risks assessment can be performed considering the endocrine disrupters properties of the substance. Nevertheless, according to elements described in the present AfA, it may be considered that:

- The environment exposure is considered to be extremely low (below the water framework directive threshold) and **near zero;**
- **Under Use-1 are included all the gel electrophoresis references manufactured and commercialized by SEBIA, representing around 30% of the global Company's turnover.** In this context, monetised impact of the "non use" scenario (loss of revenues and loss of employment) is estimated at around **€ 1.5B** while non-monetised impacts of the "non use" scenario include the loss of markets linked to a knock-off effect, relocation costs, impacts on the society health system;
- **The ban on using the substance will thus lead to the cease of production of the range and more extensively, to the bankruptcy of the Company.** It would thus result in the definite cessation of its activities and consequently, in the layoff of all the employees of SEBIA and its subsidiaries.

Consequently, benefits of continued use for SEBIA Company and the global health system outweigh widely the risk.

AoA – SEA IN A NUTSHELL

APPLICATION FOR AUTHORISATION

APPLICANT: SEBIA

USE: **Use-2**

SUBSTANCE: 4-(1,1,3,3-Tetramethylbutyl)phenol, ethoxylated

Industrial use of 4-tert-OPnEO in the production of electrophoresis gels in view of ensuring the positioning of specific proteins necessary for the interpretation of results of *in vitro* diagnostic test based on protein separation

ANALYSIS OF ALTERNATIVES

The main problematics for SEBIA regarding this type of products are the maintenance of the analytical and clinical performances and consequently, all the validation processes carried out both internally and externally, including that performed by the clients and the compulsory regulatory compliance in the event of a change of at least one device component or reagent formulation.

In this context, a preliminary internal work of research is currently carried out under easier process conditions and first results allow to consider one potential alternative to 4-(1,1,3,3-Tetramethylbutyl)phenol, ethoxylated : Tween® 20. However, considering that all analytical and clinical test performances are mainly linked to the ability of the substance to an efficient migration of proteins of interest and the immobilization of undesired macromolecules in the gel, and according to the high level of technical difficulties in keeping similar performances than those obtained with Triton® X-100, it will probably not be possible to confirm that this candidate is at least as reliable and efficient.

Consequently, as for now, no truly suitable alternative has been yet identified and is able to be developed, industrialised and qualified before the sunset date of 4-(1,1,3,3-Tetramethylbutyl)phenol, ethoxylated and a **twelve-year review period** is needed to achieve substitution.

SOCIO-ECONOMIC ANALYSIS

No benefits/risks assessment can be performed considering the endocrine disrupters properties of the substance. Nevertheless, according to elements described in the present AfA, it may be considered that:

- The environment exposure is considered to be extremely low (below the water framework directive threshold) and **near zero;**
- **HYDRAGEL® products concerned by Use-2 represents 65% of all HYDRAGEL® and INTERLAB-equivalent products, and near 30% of the global Company's turnover.** As an exact picture of the situation described in this document (and as presented in introduction of this section), the socio-economic impacts have been assessed against the 102 assays out of the 133 total tests constituting the gel electrophoresis range. In this context, monetised impact of the "non use" scenario (loss of revenues and loss of employment) is estimated at around **€ 1.5B** while non-monetised impacts of the "non use" scenario include the loss of markets linked to a knock-off effect, relocation costs, impacts on the society health system.

Consequently, benefits of continued use for SEBIA Company and the global health system outweigh widely the risk.

AoA – SEA IN A NUTSHELL

APPLICATION FOR AUTHORISATION

APPLICANTS: SEBIA

USE: **Use-3**

SUBSTANCE: 4-(1,1,3,3-Tetramethylbutyl)phenol, ethoxylated

Industrial use of 4-tert-OPnEO for its detergent properties resulting in cellular lysis and protein interactions rupture and required for the production of reagents involved in the determination of proteins of interest in IVD tested in the determination of proteins of interest

ANALYSIS OF ALTERNATIVES

The main problematics for SEBIA regarding gel and capillary electrophoresis products are the maintenance of the analytical and clinical performances and consequently, all the validation processes carried out both internally and externally, including that performed by the clients and the compulsory regulatory compliance in the event of a change of at least one device component or reagent formulation.

In this context, a preliminary internal work of research is currently carried out under easier process conditions and first results allow to consider two potential alternatives to 4-(1,1,3,3-Tetramethylbutyl)phenol, ethoxylated : Tween® 20 and Brij® 35, as a second option. However, considering that all analytical and clinical test performances are mainly linked to the ability of the substance to offer efficient lysis of cell membranes and extraction of proteins of interest kept in their native form, and according to the high level of technical difficulties in maintaining similar performances than those obtained with Triton® X-100, it will probably not be possible to confirm that these candidate are at least as reliable and efficient.

Consequently, as for now, no truly suitable alternative has been yet identified for both gel and capillary electrophoresis ranges and is able to be developed, industrialised and qualified before the sunset date of 4-(1,1,3,3-Tetramethylbutyl)phenol, ethoxylated and a **twelve-year review period** is needed to achieve substitution.

SOCIO-ECONOMIC ANALYSIS

No benefits/risks assessment can be performed considering the endocrine disrupters properties of the substance. Nevertheless, according to elements described in the present AfA, it may be considered that:

- The environment exposure is considered to be extremely low (below the water framework directive threshold) and **near zero;**
- **HYDRAGEL® and CAPILLARYS® products concerned by Use-3 represent a large part of the global Company's turnover.** As an exact picture of the situation described in this document (and as presented in introduction of this section), the socio-economic impacts have been assessed against CAPILLARYS® assays impacted by the use of the substance and for the 19 HYDRAGEL® assays out of the 133 total tests constituting the global gel electrophoresis range. In this context, monetised impact of the "non use" scenario (loss of revenues and loss of employment) is estimated at around **€ 465M** while non-monetised impacts of the "non use" scenario include the loss of markets linked to a knock-off effect, relocation costs, impacts on the society health system.

Consequently, benefits of continued use for SEBIA Company and the global health system outweigh widely the risk.

AoA – SEA IN A NUTSHELL

APPLICATION FOR AUTHORISATION

APPLICANT: SEBIA

USE: **Use-4**

SUBSTANCE: 4-Nonylphenol, branched and linear, ethoxylated

Industrial use of 4-NPnEO in the production of IVD tests in view of ensuring the positioning of specific proteins necessary for the interpretation of test results based on the determination of isoenzymes

ANALYSIS OF ALTERNATIVES

The main problematics for SEBIA regarding this type of products are the maintenance of the analytical and clinical performances and consequently, all the validation processes carried out both internally and externally, including that performed by the clients and the compulsory regulatory compliance in the event of a change of at least one device component or reagent formulation.

In this context, an preliminary internal work of research is currently carried out under easier process conditions and first results allow to consider one potential alternative to 4-Nonylphenol, branched and linear, ethoxylated: Tween® 20. However, considering that all analytical and clinical test performances are mainly linked to the ability of the substance to offer to an efficient migration of proteins of interest and the immobilization of undesired macromolecules in the gel, and according to the high level of technical difficulties in keeping similar performances than those obtained with 4-Nonylphenol, branched and linear, ethoxylated, it will probably not be possible to confirm that this candidate is at least as reliable and efficient.

Consequently, as for now, no truly suitable alternative has been yet identified and is able to be developed, industrialised and qualified before the sunset date of 4-Nonylphenol, branched and linear, ethoxylated and a **seven-year review period** is needed to achieve substitution.

SOCIO-ECONOMIC ANALYSIS

No benefits/risks assessment can be performed considering the endocrine disrupters properties of the substance. Nevertheless, according to elements described in the present AfA, it may be considered that:

- The environment exposure is considered to be extremely low (below the water framework directive threshold) and **near zero;**
- As an exact picture of the situation described in this document (and as described in introduction of this section), the socio-economic impacts have been assessed against the 3 assays out of the 133 total tests constituting the global HYDRAGEL® range. In this context, monetised impact of the "non use" scenario (loss of revenues and loss of employment) is estimated at around **€ 4.3M** while non-monetised impacts of the "non use" scenario include the loss of markets linked to a knock-off effect, relocation costs, impacts on the society health system.
- HYDRAGEL® products concerned by Use-4 represent around **1% of the global Company's turnover**, which can be considered quite low. **Nevertheless, considering the nature of diseases diagnosed with these kits, and the near-monopoly of SEBIA in this assays area, social and mainly, medical impacts of the ban on using the substance could be highly critical.**

Consequently, benefits of continued use for SEBIA Company and the global health system outweigh widely the risk.

6.3. Information for the length of the review period

Given the argument put forward, and in order to develop, implement and validate alternatives for Use-1, SEBIA applies for a twelve-year review period, considering that in the case of the discontinuation of the HYDRAGEL® range, SEBIA could have to file for bankruptcy.

6.4. Substitution effort taken by the Applicants if an authorisation is granted

If an authorisation is granted, SEBIA will pursue the substitution process described in section 4.2.

7. ANNEX – JUSTIFICATIONS FOR CONFIDENTIALITY CLAIMS

Confidential information was blanked out in the public version in order to preserve the confidentiality of strategic data of the present AfA.

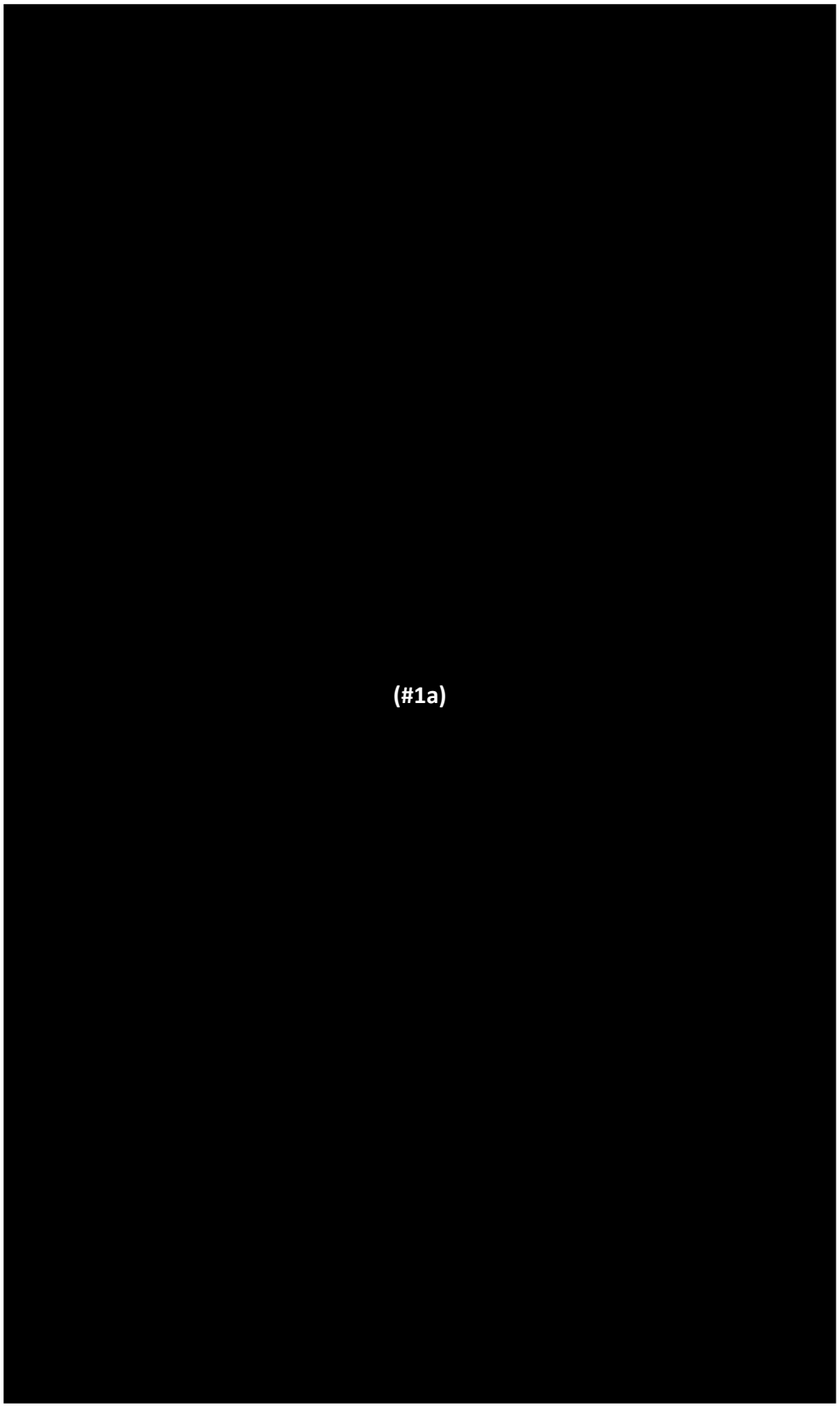
The following table provides a justification for confidentiality of the blanked-out data of this document.

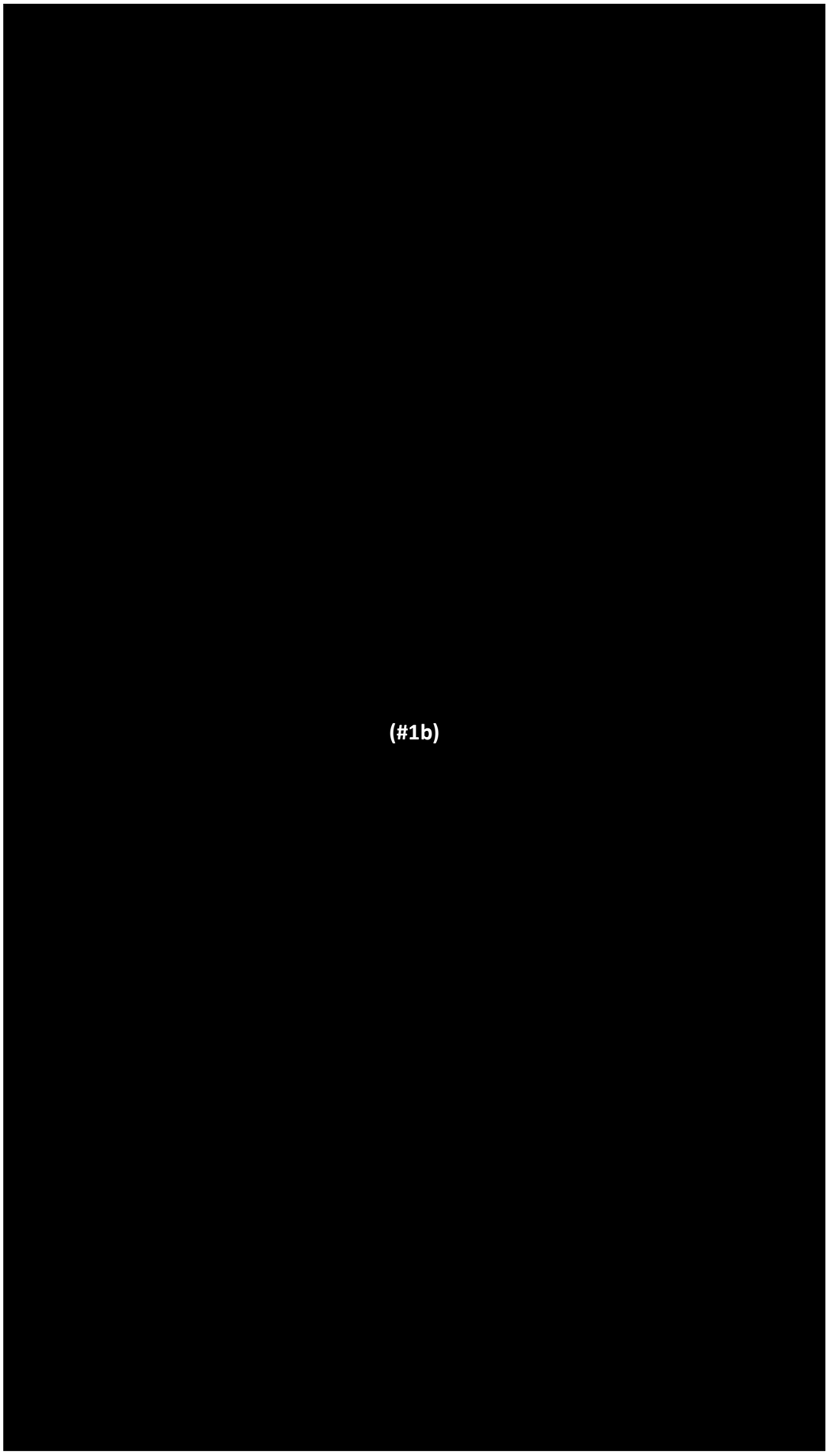
BLANKED OUT ITEM REFERENCE	PAGE NUMBER	JUSTIFICATION FOR CONFIDENTIALITY
#1	101, 102, 103, 104	**Strategic data**: the blanked data concern non-public market figures characterising the activity of SEBIA.

Table 39. Justifications for confidentiality claims

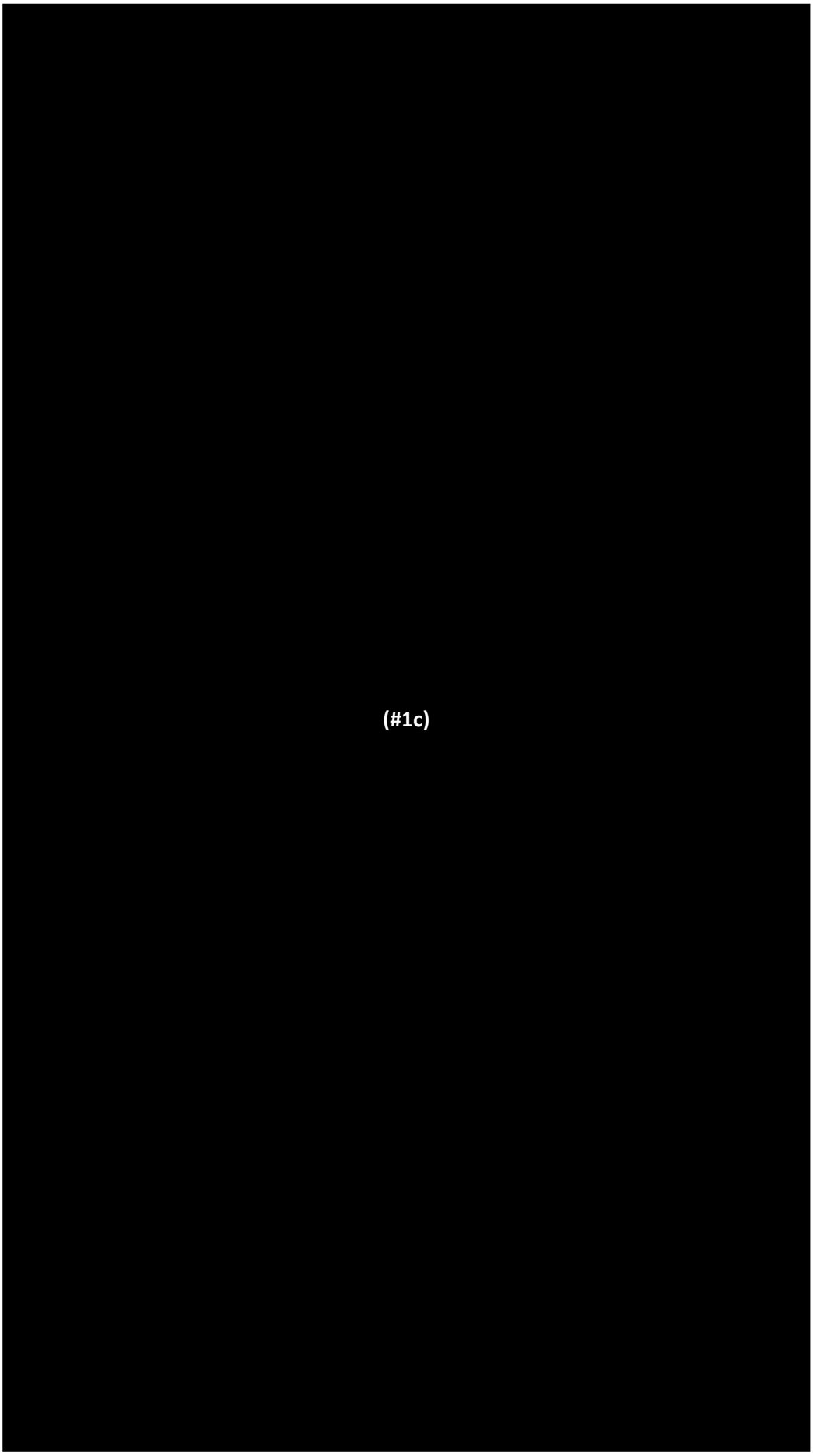
8. APPENDIXES

8.1. Appendix 1: Number of Use-1 kits sold and number of patients diagnosed in 2017

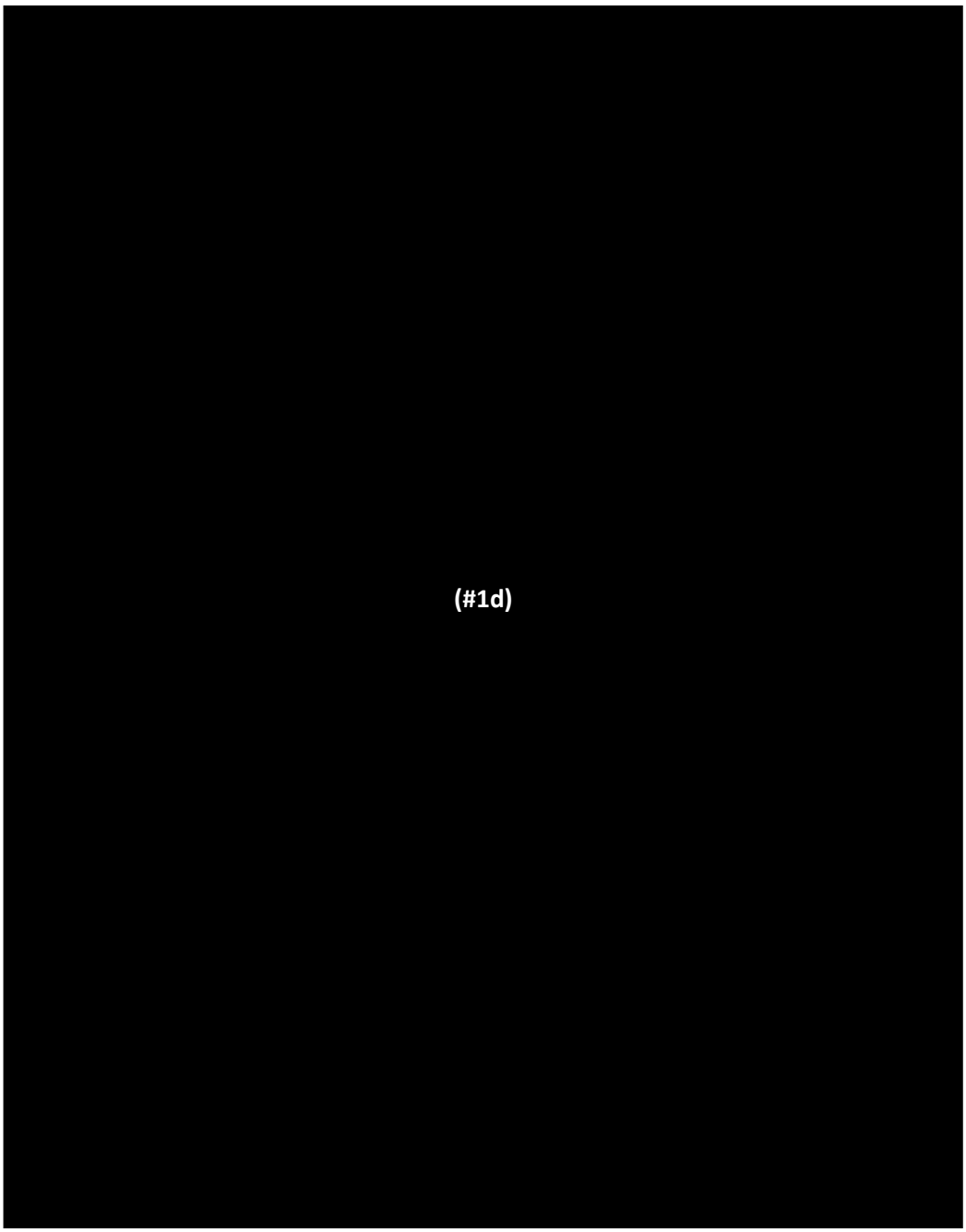




(#1b)



(#1c)


(#1d)

8.2. Appendix 2: Example of MSDS including a special notice about the presence of the substance in the kit

SAFETY DATA SHEET

I – COMPANY AND PRODUCT IDENTIFICATION

Company name	:	SEBIA
Address	:	Parc Technologique Léonard de Vinci - Rue Léonard de Vinci CP 8010 Lisses 91008 EVRY CEDEX - FRANCE
Phone number	:	33.(0)1.69.89.80.80
Fax number	:	33.(0)1.69.89.78.78
E-mail	:	sebia@sebia.com
Product name	:	**HYDRAGEL 3 & 9 CSF ISOFOCUSING**
Product reference	:	4353 / 4355
Type of product	:	*For in vitro diagnostic use*

Contains substances subject to authorization (Entries 42 and 43 of Annex XIV) endocrine disrupters with an impact on the environment (sections 3-12-13-15 of the SDS)

Emergency call number : + 844 892 0111 (National Poisons Information Service (NPIS))

II – KIT CONTENTS

HYDRAGEL 3 & 9 CSF ISOFOCUSING gel

CSF ISOFOCUSING anodic solution

ISOFOCUSING cathodic solution

CSF/A1AT/TRF sample diluent

Ethylene-glycol solution

CSF ISOFOCUSING antiserum diluent

CSF ISOFOCUSING wash solution

Rehydrating solution

TTF1 – TTF2 solvent

TTF1

TTF2

Data contained in this data sheet is based on information available at the date of issue. This information cannot be considered as exhaustive and will in no case relieve product users to obtain information from other sources in order to ensure for correct use of the product and staff safety. SEBIA cannot be held liable to any damage resulting from use or from contact with the product described in this data sheet.

Reference: SDS/CIA/SEB/4353 Revised on: 2019.07.01

8.3. Appendix 3: Example of website content

Entered into force on 1st June 2007, REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) is a European Union regulation adopted to better protect human health and the environment from the risks associated with chemical substance while promoting the competitiveness of the EU chemical industry. Among the different REACH procedures, the purpose of the Authorisation procedure is to ensure that substances of very high concern (SVHC) are progressively replaced by other less dangerous substances or technologies when economically and technically viable alternatives are available. These dangerous substances are then subject to successive regulatory procedures until they are included in Annex XIV prohibiting their use at a given date. Thus, unless you benefit from an Authorisation granted by the European Commission, it is no longer possible to use the substance concerned. This Authorisation is then granted for a limited period of time but considered sufficiently long for the recipient company to identify and develop an alternative solution.

In this context, SEBIA is already committed to removing any potential or proven SVHC from future developments. To do this, SEBIA ensures an intensive regulatory watch to anticipate any inclusion in Annex XIV of one of the substances included in its production schedule.
For the substances already concerned, SEBIA implements the conditions necessary to control the impacts related to its activities and to secure its value chain, in particular by:

- A reasoned management of its waste with a goal of "zero rejection";
- The filing of authorization application files for as short a time as possible in order to replace the offending substance as quickly as possible and covering both its direct suppliers and its user customers;
- Strengthening its communication on the products concerned through regular training sessions on the use of SEBIA products, writing FDS detailing all the risk management measures to be implemented, publishing specific explanatory materials, ...

Recently, 4-(1,1,3,3-Tetramethylbutyl) phenol, ethoxylated (Triton ™ X-100, Igepal® CA-630, ...) and 4-nonylphenol, ethoxylated (Nonidet® NP-40, Tergitol ™ NP, ...) detergents have been incorporated into the Annex XIV for endocrine disruptor properties whose impact on the environment is now demonstrated. SEBIA is therefore actively seeking compounds with equivalent performance in order to maintain the quality of its products and to disseminate best practices for the use of electrophoresis kits in controlled conditions. In this context, the MSDS of products containing these detergents have been updated.

For the conditions and rules of good practice for the use of kits containing these detergents, please consult the MSDS of the kits concerned.

8.4. Appendix 4: Example of information brochure aimed at end-users

Since 2007, the European REACH Regulation aims to better protect human health and the environment from the risks associated with chemical substances, while promoting the competitiveness of the EU chemical industry. In order to better meet the requirements of the regulations, SEBIA is committed to:

- Remove all hazardous substances from its kits and production processes;
- Develop, in this context, alternative processes and substitutes for dangerous substances;
- Implement and disseminate throughout its value chain all safety conditions for user personnel and environmental risk management measures by promoting, among other things, an optimized waste management policy aimed at "zero rejection".

Through the application of these three actions, SEBIA is working to secure the entire upstream and downstream activities of the IVD sector. The case of O / NPE detergents recently identified as substances of very high concern (SVHC) is an example. The set of good practices of use can be consulted on the SDS of the electrophoresis kits concerned.