

## **ANALYSIS OF ALTERNATIVES**

**Legal name of applicant(s):** Roche Diagnostics GmbH

**Submitted by:** Roche Diagnostics GmbH

**Substance:** Diglyme: Bis(2-methoxyethyl)ether  
(EC Number 203-924-4; CAS Number 111-96-6)

**Use title:** Use of Diglyme as a process chemical in the manufacture of one specific type of Dynabeads® used in immunodiagnostic assays (IVD)

**Use number:** 1

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## GLOSSARY

Term	Explanation
<b>Biomolecules</b>	Polypeptides (peptides, antibodies, streptavidin, enzymes) and nucleic acids.
<b>Coating</b>	An epoxide or polyurethane type material that provides: (a) a surface with characteristic balance between hydrophobic and hydrophilic properties; (b) filling media for the inherent pores of the particles; (c) a seal to keep the iron particles within the beads; and (d) a reaction surface for functionalization of the beads to bind biomolecules.
<b>Conjugation</b>	<p>Production step through which biomolecules are chemically attached to the bead. The “attachment” of biomolecules can be both of non-covalent nature (physical adsorption onto the surface) or covalent nature (attached with a chemical bond).</p> <p>A word that is often used interchangeably to conjugation is “immobilization.”</p>
<b>Dynabeads®</b>	<p>Superparamagnetic spherical polymer particles with a uniform size and a consistent, defined surface for the adsorption or coupling of various bioreactive molecules or cells. They can be used for numerous biochemical and medical applications. (Patent for example EP1693387).</p> <p>In 2017, RDG will be producing a specific type of Dynabeads® as well as continuing to be supplied by Life Technologies AS. The beads will be used in RDG Elecsys® assays.</p>
<b>Elecsys® assay</b>	<p>Elecsys® assays are <i>in vitro</i> diagnostic assays that cover a wide range of testing parameters which run on Roche Elecsys® instruments. These immunoassays are dependent on one type of Dynabeads® beads for the proper functioning. The Elecsys® system includes the instruments, assays, and software.</p> <p>Per assay in this portfolio, a so-called rack pack is put together containing three “cartridges”, which consist of the following:</p> <ul style="list-style-type: none"> <li>• One cartridge contains Dynabeads®; and</li> <li>• Two cartridges containing two antibodies and additional ingredients necessary for the assay.</li> </ul>
<b>Functionalization</b>	Introducing reactive groups, reactive chemistry augmented to the coat to enable conjugation of the biomolecules.


<b>IVD</b>	<p><i>In vitro</i> diagnostic medical devices. IVD products are regulated and defined by Directive 98/79/EC, meaning any medical device which is a reagent, reagent product, calibrator, control material, kit, instrument, apparatus, equipment, or system, whether used alone or in combination, intended by the manufacturer to be used <i>in vitro</i> for the examination of specimens, including blood and tissue donations, derived from the human body, solely or principally for the purpose of providing information:</p> <ul style="list-style-type: none"> <li>▪ Concerning a physiological or pathological state, or</li> <li>▪ Concerning a congenital abnormality, or</li> <li>▪ To determine the safety and compatibility with potential recipients, or</li> <li>▪ To monitor therapeutic measures.</li> </ul>
<b>Magnetic beads</b>	Polystyrene based particles with iron embedded. The paramagnetic properties of the beads allow the use of a magnet to separate the beads from the liquid milieu (downstream use). Dynabeads® are one type of magnetic beads that are considered superior due to their uniformity and quality.
<b>Porous polystyrene beads</b>	Porous spherical polymer particles made from styrene type monomers.
<b>Polystyrene beads with iron oxide but no coating</b>	Magnetic iron oxide is precipitated into the pores of polystyrene beads.
<b>Polystyrene beads with embedded iron oxide and coated</b>	Iron oxide is sealed into the pores of polystyrene beads by adding a coating of epoxy or polyurethane dispersed in a solvent.
<b>Purification</b>	Removing excess reactants and solvents up to a concentration where there is no impact on subsequent reaction steps.
<b>Roche Diagnostics GmbH (RDG)</b>	Part of the diagnostic division of F. Hoffmann-La Roche Ltd. Roche Diagnostics GmbH (RDG) has an extensive portfolio, one aspect of which is the manufacturing of instrument platforms and reagents for the different Roche affiliates worldwide. It is located in Germany (Mannheim and Penzberg).
<b>(Re-)validation</b>	Any change to the production process that could change the form, fit or function of the beads (including moving production to a new facility) must be validated. The purpose of the validation is to assess that the performance characteristics of the products have not been impacted by the changes to the production process.

DECLARATION

We, Roche Diagnostics GmbH (RDG), request that the information blanked out in the “public version” of the Analysis of Alternatives is not disclosed. We hereby declare that, to the best of our knowledge as of today (17.02.2016) the information is not publicly available, and in accordance with the due measures of protection that we have implemented, a member of the public should not be able to obtain access to this information without our consent or that of the third party whose commercial interests are at stake.

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## 1. SUMMARY

Roche Diagnostics GmbH (RDG) is applying for an authorization to use diglyme to support the planned future production of one specific type of Dynabeads®. This specific type of Dynabeads® is currently supplied by Life Technologies AS (LT) and further conjugated at RDG's site in Penzberg. These Dynabeads® are an essential component of the whole Roche Elecsys® portfolio and are manufactured using diglyme, a unique dipolar aprotic solvent. The critical physical and chemical compatibility properties of diglyme are essential to the coating reaction and the purification of the magnetic beads.

Magnetic beads must be able to bind to streptavidin (conjugation step), with no residual chemicals or solvents remaining from the manufacturing process. Any residual solvent/impurity can result in false negative or false positive signals when the Elecsys® assay is run. The solvent used must not impact critical performance parameters (diameter and specific surface area). In the past, several beads were tested to obtain a market overview of potential alternative beads (instead of Dynabeads®), but none of the beads were, as such, suitable for the use in Elecsys® assays.

At the moment, RDG has no experience in producing Dynabeads®, and hence has not had the opportunity to conduct research into alternative solvents for this process. RDG, however, as part of the Roche Group is publically committed to substituting any Substances of Very High Concern (SVHC) from their processes and products.

This Analysis of Alternatives (AoA) focuses on RDG's role in evaluating the performance of the Dynabeads® produced by LT with alternative solvents to diglyme. The application explains the unique technical and regulatory challenges associated with validating an alternative to diglyme. A 12-year Authorisation review period will allow RDG, in collaboration with LT, to develop, evaluate, validate, and if necessary, submit change notifications as a regulatory requirement for *in vitro* diagnostic assays<sup>1</sup>. Millions of patients worldwide depend on the accurate, reproducible and reliable results of the Elecsys® assays, which in turn depend on this one specific type of Dynabeads®.

## 2. INTRODUCTION

Roche Diagnostics GmbH (RDG) is a subsidiary of F. Hoffmann-La Roche Ltd. (Roche). Roche was founded in 1896 by Fritz Hoffmann-La Roche, who was among the first to recognize that the industrial manufacture of standardized medicines would be a major advance in the fight against disease. From its inception, the company has focused on innovation and on establishing an international presence as an innovator in the field of healthcare. Roche is currently one of the world's leading, research-oriented healthcare companies and has two core businesses: pharmaceuticals and diagnostics.

RDG is the second largest subsidiary within Roche and one of the biggest employers in upper Bavaria. It is located in Mannheim and Penzberg. The latter site is one of the biggest biotech centers in Europe and a leading biotechnology research, development and production site in the world.

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<sup>1</sup> Please note that 12 years will not be enough to cover completely new market authorisation submissions worldwide for Elecsys® assays in case of changes in the bead properties.

RDG's main objective is to discover and develop innovative diagnostic solutions that can be used by health-care professionals as an integral part in decision-making along the entire continuum of a patient's health or disease. *In vitro* diagnostic tests influence more than 60% of clinical decisions providing health-care professionals with high-value, actionable results that can be used to prevent, manage and treat diseases more effectively.

A large number of reagents and ingredients for the Roche diagnostic systems are produced at the Penzberg site. The broad diagnostics technology and product portfolio is unique in the world and ranges from immunoassays, for example to detect heart failure or prostate cancer; through enzyme assays, for example to measure cholesterol or glucose; to research reagents and industry products. Over the last decade Roche has invested around 1.8 billion euros in expanding the Penzberg Biotechnology site.

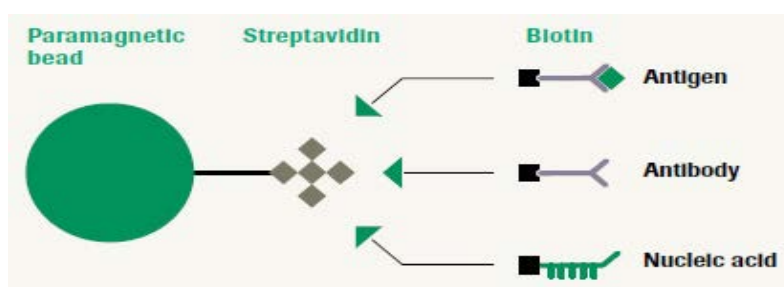
There are around 100 Elecsys® assays for detecting and quantifying markers for different diseases/conditions. These applications are used in over 33,000 active systems worldwide, providing a total test volume of 1.6 billion tests per year (50 patient results per second).

The Elecsys® assays use ElectroChemiLuminescence (ECL) ([video](#)), a unique immunoassay detection technology.

**Immunoassays function on the principle that the marker (antigen) in the sample (e.g., blood) is detected using an antibody that is specific for this marker.**

The magnetic beads are an essential element of Elecsys® immunodiagnostic assays, separating the antibody-antigen complex from the rest of the sample to enable quantification of the marker. Before use in the Elecsys® system, the magnetic beads are further processed by RDG. The purpose of this further processing is to cover magnetic beads with the protein streptavidin<sup>2</sup> (**conjugation step**). This step is crucial for the binding of the antibody-antigen complex to the bead which is essential for its isolation (see figure 1).

**Figure 1: Magnetic bead conjugated with streptavidin to capture biotin**



<sup>2</sup> Streptavidin is a protein with an extraordinarily high affinity for biotin. The binding of biotin to streptavidin is one of the strongest non-covalent interactions known in nature. Streptavidin is used extensively in molecular biology due to the streptavidin-biotin complex's resistance to organic solvents.

All assays depend on the use of one specific type of Dynabeads®. Extreme consistency within and between batches of beads is of utmost importance for the reproducibility of the assays. This consistency is the result of close collaboration, over the past 20 years, with the supplier of these magnetic beads, LT<sup>3</sup>.

Recently, RDG decided to invest in a technology transfer from LT for **its own production of Dynabeads® at the Penzberg<sup>4</sup> site. These specific beads, a core part of the whole Elecsys® assay portfolio, are produced using the solvent diglyme.**

RDG is applying for an authorization for the future use of diglyme to support the planned production of one specific type of Dynabeads® in Penzberg.

The production of magnetic beads is a complex, multi-step process to **build a micro size polymeric bead structure, containing magnetic iron oxide**, which is able to bind streptavidin.

**Bis(2-methoxyethyl)ether (Diglyme) (EC Number 203-924-4; CAS Number 111-96-6) has a critical role in the coating and purification of the beads.** Diglyme is a dipolar aprotic solvent with unique physico-chemical properties (e.g. boiling point >100°C) making it compatible with chemicals used in the Dynabeads® manufacturing process.

Magnetic beads must be able to bind to streptavidin, with no residual chemicals or solvents remaining from the manufacturing process. Any residual solvent/impurity can result in false negative or false positive signals when the Elecsys® assay is run. The solvent used must not impact this critical performance parameter.

The **diameter and specific surface area** of the bead coating are **critical main parameters** that **impact the degree of surface functionalization** of the beads, which in turn affects the quantity and **consistency of the bead binding to streptavidin** and its end use.

Diglyme is classified as toxic for reproduction 1B, H360FD (“May damage fertility. May damage the unborn child”). Diglyme was prioritized for inclusion in Annex XIV of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) Regulation in the European Chemical Agency’s (ECHA) 4th Recommendation and formally added to Annex XIV (under entry 25) with a sunset date of 22 August 2017. In accordance with the regulatory framework, the substance cannot be placed in the European Union (EU) market or used (after the sunset date), unless an authorization has been granted.

### 3. ANALYSIS OF SUBSTANCE FUNCTION

The Elecsys® system was first launched in 1996. Over the past 20 years, RDG has been using one specific type of magnetic beads (Dynabeads®) produced by LT as an essential component of the reagents used in the entire Elecsys® product series. This specific type of Dynabeads® is a polyurethane-coated bead.

LT is the only supplier in Europe and worldwide of this specific type of magnetic bead. Dynabeads® are monosized particles, produced only in Lillestrøm and supplied to RDG (Penzberg)

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<sup>3</sup> LT has also decided to apply for an authorisation for the use of Diglyme as a process chemical in the manufacture of Dynabeads® range.

<sup>4</sup> See Socio-Economic Analysis (SEA) -- 2.6 Definition of the applied for use scenario.



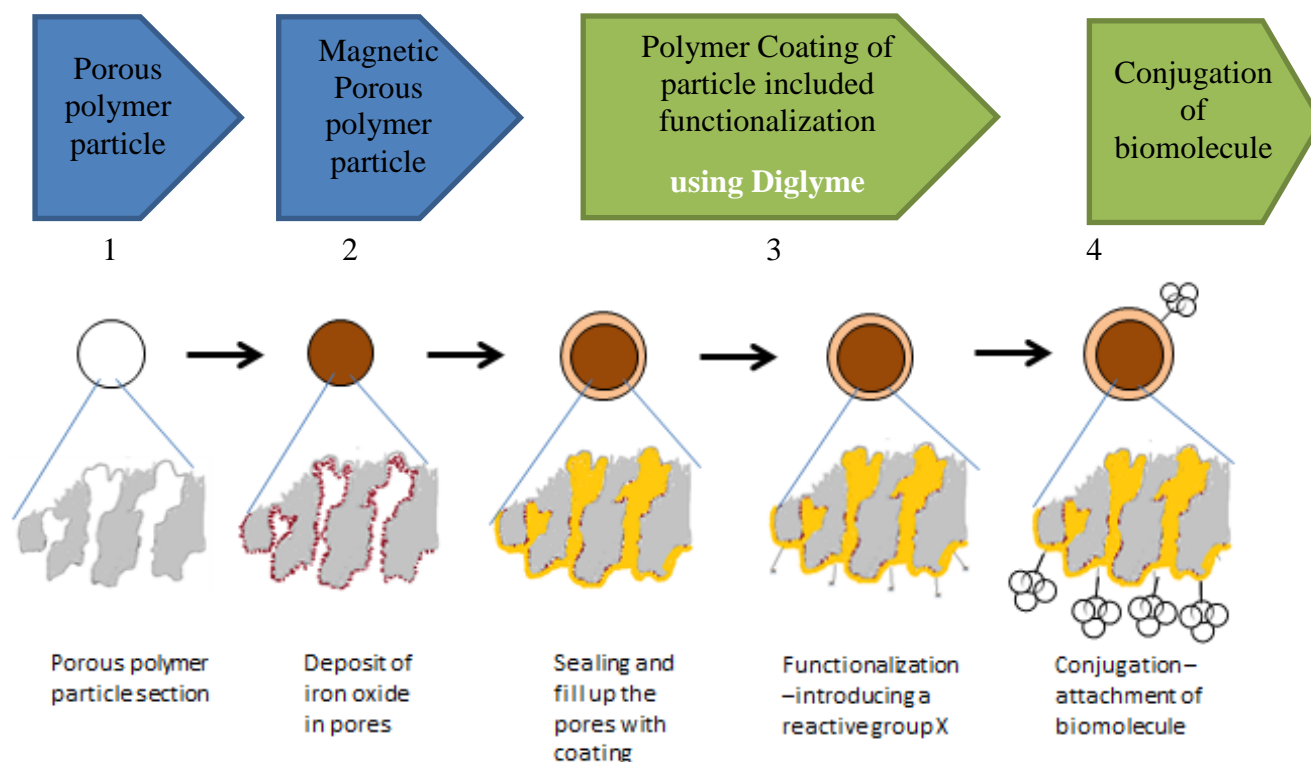
and suspended in water to be further processed (**conjugation step**) before they can be used in the Elecsys® assays.

**RDG made the decision to invest in a technology transfer from LT and launch its own production of Dynabeads® in Penzberg.** The production of magnetic beads is expected to start in 2017.

LT will, nevertheless, continue to supply an amount of the beads as per the license agreement, and all conjugation steps with streptavidin will still be made in Penzberg for all the beads.

### 3.1. Description of the Entire Future Production Process of the Conjugated Beads for Use in Elecsys® Assays

**Figure 2: Illustration of the multistep process**



The basic process will be as follows:

After each step, washing with the relevant solvent will be performed before moving to the next step. The relevant wash solvents are water, diglyme and a ketone (to remove diglyme):

1. A porous polymer particle will be made from styrene type monomers.
2. Magnetic iron oxides will be precipitated into the pores and are, prior to the next step, washed with water then with diglyme for the coating step.

3. The iron oxides will be then be sealed into the pores by polyurethane **coating** covering the surface of the particle to:
  - a) Fill the pores;
  - b) Prevent leakage of iron oxide from within the particle;
  - c) Make the polystyrene surface more hydrophilic; and
  - d) Introduce chemical groups that could be used for further chemical modifications or binding of streptavidin.

Prior to conjugating the biomolecule onto the particle, beads are **purified** with diglyme then with acetone to remove diglyme. They are dried and suspended in water.
4. **Conjugation** is performed with streptavidin.

### 3.2. Diglyme Function in the Production Process

There are 2 steps in which diglyme is critical for the production of the Dynabeads®.

#### Coating

The **coating process is a critical element** of the Dynabead® production, as this step of the patented and trade secret based technology distinguishes the bead from its competitors. Polystyrene beads are coated using diglyme as the key solvent in the process. To enable this coating, the beads are suspended in diglyme.

To prepare for the coating process, polyurethane precursor monomers are added to the beads suspended in diglyme. The chemicals react with anchoring groups on the surface of the bead and on the surface of the pores inside the bead. The coating reaction fills up a large part of the pore volume. The surface area of the porous polymer beads in the coating reaction typically changes from several hundred m<sup>2</sup>/gram particles before coating to less than 10 m<sup>2</sup>/gram particles after coating. The **specific surface area and composition of the coating determines the amount of streptavidin** that can be bound to the bead surface by conjugation.

Experiments have shown that diglyme critically affects the reaction kinetics and the solubility of the polymers formed during the reactions, which are essential for the proper polymerization of the coating on the bead. Furthermore, the **coating thickness affects directly the specific surface area and the functionality** of the bead in the final diagnostic applications.

**Many organic solvents may be chemically incompatible with the magnetic bead coating processes. Diglyme was established as a versatile solvent for the reactions used to produce Dynabeads® because diglyme is chemically compatible with all the coating reactants. Use of diglyme avoids several classical interference and unwanted side reactions.**

#### Purification

After the coating reaction, **diglyme is used in the purification of the beads** to remove excess reactants to a concentration where there is no impact on subsequent reaction steps. The beads are purified by magnetic separation. This is done sequentially, several times using diglyme as the solvent to remove excess monomers and polymer not covalently attached to the beads. The **purified magnetic beads are then washed sequentially several times in acetone to remove diglyme** and also to prepare for further operations.

#### 4. ANNUAL TONNAGE

Under the License Agreement, LT will continue to manufacture █ kg of beads each year for RDG's use. As part of the terms, RDG will manufacture in the future █ kg<sup>5</sup> each year<sup>6</sup> and, for this quantity, it is envisioned that 2 tonnes of diglyme per year will be required to produce this one specific type of Dynabeads®. However, given the increasing demand for Dynabeads® and on the basis of the overall capacity of the plant being built in Penzberg, RDG is applying for an authorization to use up to 8 tonnes of diglyme per year.

#### 5. IDENTIFICATION OF POSSIBLE ALTERNATIVES

The applicant has looked at the possibility of using alternative beads or finding an alternative solvent to diglyme.

##### 5.1. Looking for Alternative Beads to Dynabeads®

In the past, **several beads were tested to obtain a market overview of potential alternative beads to Dynabeads®** and to test whether such alternatives may provide better performance than the beads currently in use. As the beads were purchased from different suppliers for testing, information on the solvents used in the production of the beads was not available at the time of testing.

**None of the beads fulfilled the specifications defined for Dynabeads®.** In particular, none of the beads were functionalised with the same functional group as Dynabeads®. Furthermore, RDG is not aware of such beads on the market. Based on the delivery contract, RDG is not allowed to further communicate details on Dynabeads® and, therefore, cannot suggest the specific functionalisation to other suppliers.

During the testing, **different conjugation procedures** (*i.e.*, covalent or non-covalent binding of the streptavidin) **were evaluated** if technically required. **None of the beads were as such suitable for the use in Elecsys® assays**, mostly due to low signal dynamics.

The results of the tests thus underline the difficulty in identifying a technically feasible alternative bead. Table 1 provides examples of alternative beads tested, including a short description of why the beads were not suitable.

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<sup>5</sup> █ kg is the expected average quantity that will be produced by RDG. However, the production might range from █ kg to █ kg per year (depending on market development and also on the production schedule).

<sup>6</sup> 1-2 operators will be needed in case Penzberg produces the average █ kg. In the worst case scenario, *i.e.* using 8 tons of diglyme, 6-7 employees will be hired.

**Table 1: Alternative beads tested during the past decade**

<b>Microparticle</b>	<b>Manufactured by Company</b>	<b>Year of Testing</b>	<b>Signal Dynamics (% of reference)</b>	<b>Summary of Assessment</b>
Particle 1	S	2007	23	not suitable, low signal dynamics, new conjugation procedure necessary
Particle 2	P	2007	1	not suitable, low signal dynamics, new conjugation procedure necessary
Particle 3	M	2011	n.d.	not suitable, low signal dynamics, new conjugation procedure necessary
Particle 4	M	2007	n.d.	not suitable, broad particle size distribution
Particle 5	B	2007-2010	1	not suitable, low signal dynamics, new conjugation procedure necessary
Particle 6	H	2007	n.d.	not suitable, very low biotin binding capacity
Particle 7	P	2007-2008	70	not suitable, new conjugation procedure necessary, high lot to lot variability
Particle 8	T	2010	50	not suitable, low signal dynamics
Particle 9	T	2010	52	not suitable, low signal dynamics
Particle 10	T	2010	60	not suitable, low signal dynamics
Particle 11	T	2010	5	not suitable, low signal dynamics, new conjugation procedure necessary
Particle 12	T	2010	29	not suitable, new conjugation procedure necessary
Particle 13	T	Jul 05	94	suitable for Elecsys® analyzers; new conjugation procedure necessary

Among the beads tested, only one bead, particle 13, was, in principle, suitable for the use in the Elecsys® assays. However, **to use this bead, the conjugation procedure performed in Penzberg would need to be adapted.** The alternative conjugation procedure was only tried at the laboratory scale and a transfer to production scale would involve a significant amount of optimisation work on the conjugation process. Whether such optimisation work would be successful and the bead would be a technically feasible alternative with the required quality and low lot-to-lot variability is questionable.

Furthermore, a change in the conjugation step requires **long and extensive internal validation of assays** (R&D and Operations) and then **regulatory work in terms of IVD market authorisation.** In the best case, if the product equivalency<sup>7</sup> is shown despite a change in process, an amendment in notification is needed for EU, U.S. and China (10 weeks of work per region plus regulatory fees). However, it is most likely that any process changes in the manufacture of the conjugated beads will lead to different parameters and equivalency will not be clearly demonstrated. In this case, new submissions (including clinical studies) will be needed for all assays and all regions. For the U.S. alone, if we assume that of the 100 Elecsys® assays, 10 are considered as highly regulated assays,

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<sup>7</sup> Product equivalency based on pre-defined acceptance criteria based on results from performance evaluation and validation studies of a group of assays that show whether the new beads have the same quality as the existing ones currently in use.

then for a new submission in the U.S. (PMA<sup>8</sup>) an estimated 60 months of regulatory work is needed, **not** including time for requested clinical studies and authority evaluation time. The new submission fees for the 10 assays in the U.S. amount to 2.5 million USD.

It needs to be emphasized that **this particular bead does not represent an alternative as it is also produced with diglyme** based on information later obtained from LT as supplier of this bead.

Even if it were possible to identify an alternative bead, the research on and use of such a bead would have a range of economic consequences:

- Part of the investment for the licence agreement would be sunk cost (the agreed duration of the licence agreement is 20 years);
- Part of the investment for the production facility for the beads would be sunk cost;
- Additional large investment would be required to find and optimise the alternative beads with uncertain outcome;
- There would be a high uncertainty regarding future reproducibility / lot-to-lot variability and consequently also economic uncertainty;
- If the conjugation process was changed, the optimised conjugation process would need to be validated in production and the production facility for the conjugation may even need to be changed as this facility is designed for the current conjugation process;
- Depending on whether the change in bead requires an adaptation of the conjugation process, a new market authorisation submission would need to be made for the different assays and different regions;

Given **the past unsuccessful testing of alternative beads**, the high uncertainty of the outcome of any investment, and the high associated cost (including loss of performed investment) on the production of Dynabeads®, **using alternative beads was found to be technically and economically infeasible** in the current situation and thus, not to be pursued.

## 5.2. Research of Alternative Solvent(s) to Diglyme

RDG is applying for authorization for a future use of diglyme and the process is not yet established at the facility in Penzberg. Therefore, RDG has not had and still does not have the ability to conduct research activities on alternative solvents. **RDG, however, plays an important role in the evaluation of beads (manufactured with an alternative solvent) for its use when potential alternative solvents are proposed/evaluated by LT.** Successful performance within RDG's Elecsys® system, particularly the appropriate binding of streptavidin, is critical and will ultimately determine whether a potential alternative solvent will allow RDG's Elecsys® system to continue to function accurately and thus dictate the feasibility of any potential alternative solvent.

LT, the manufacturer of magnetic beads and owner of the technology, launched a **feasibility study** in 2014 to identify potential alternative solvents to diglyme. As part of this evaluation, several solvents are tested in the coating reaction and purification steps with the specific purpose of **identifying any challenges of substituting diglyme.**

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<sup>8</sup> PMA: Pre-market approval.

**RDG performance evaluation** is conducted on a **test panel** designed by LT based on different precursor polystyrene beads using several potential alternative solvents in the coating reaction and purification steps. The “test-panel” is shown below in Table 2.

**Table 2: First test-panel of alternative beads designed by LT**

	Polystyrene Beads	Solvent in Reaction	Purification Solvent	Comment
1	MAG 1	Diglyme	Diglyme	Production Reference
2	MAG 1	Diglyme	Diglyme	R&D scale reference
3	MAG 1	Diglyme	B	purification
4	MAG 1	Diglyme	C	purification
5	MAG 1	A	B	Reaction + purification
6	MAG 1	A	C	Reaction + purification
7	MAG 2	Diglyme	Diglyme	Reference
8	MAG 2	Diglyme	Diglyme	R&D scale reference
9	MAG 2	A	B	Reaction + purification
10	MAG 2	A	C	Reaction + purification
11	MAG 3	Diglyme	Diglyme	Production Reference
12	MAG 3	Diglyme	Diglyme	R&D scale reference
13	MAG 3	A	B	Reaction + purification
14	MAG 3	A	C	Reaction + purification

RDG will evaluate the performance of these experimental beads manufactured using potential alternative solvents by LT. The performance testing will start in Penzberg using R&D scale samples and production reference samples (with diglyme) at this stage.

The purpose of the evaluation is to identify any potential deviations in the performance of beads manufactured using an alternative solvent as compared to diglyme. **This performance evaluation is the key element for the selection of potential alternative solvents.**

The performance evaluation of alternative beads at RDG will be similar to the standard performance evaluation of beads currently in place with the addition of a number of steps. The standard procedure, including challenges to meet bead specifications in the current production, is described in Section 5.2.1. Section 5.2.2 includes the additional steps when evaluating the beads produced with a solvent other than diglyme.

The feasibility study, including performance evaluation by Penzberg, must be performed iteratively before a comprehensive process development program with an alternative solvent is developed at LT, and may require up to 4 years. Table 3 gives an overview of the additional steps needed to be able to substitute diglyme based on the assumption that a change in solvent only leads to a small change in the beads and the conjugation process remains the same.

**Table 3: Steps required to be able to substitute diglyme**

	<b>Estimated time (months/ years)</b>	<b>Role of LT</b>	<b>Role of RDG</b>
<b>Feasibility Study</b>	1.75y – 4y	Design of potential alternative beads (test panel)	Performance evaluation of the test panel
<b>Development</b>	1.75y – 5.25y	Process development	Acceptance
<b>Validation (small change)</b>	10.5m – 1.6y	Process validation	Bead conjugation and validation in the assays
<b>Market Authorisation</b>	8.5m – 1.2y	-	Change notification (+ authority evaluation)

The different steps are described in detail in the following sections.

### 5.2.1. Standard Performance Evaluation

Bead performance evaluation is conducted at RDG as part of a standard procedure in the production process.

#### a) Technical specifications

The supplier ensures that the following technical specifications for the coated beads are met (acceptable values are described in the specification sheet for Dynabeads®):

- Concentration of beads;
- Bead size (diameter);
- Size distribution;
- Magnetic susceptibility;
- Specific surface area; and
- Bioburden (germ number).

Before accepting a complete delivery of beads and launching large-scale conjugation of the batch with streptavidin, RDG tests samples of each batch in the production facility in Penzberg. This is defined in a testing procedure for coated beads before conjugation. This includes an optical examination of the received coated beads, germ number determination and checking of specifications based on the parameters provided by LT (arrival inspection). The test batch is then conjugated and the **conjugated bead sample is also further tested based on the performance parameters** described below.

The technical specification parameters of the conjugated beads are:

- Appearance;
- Concentration;
- Free streptavidin (verification of binding of streptavidin on the surface);
- Specific biotin binding capacity;
- Bioburden (germ number); and
- Function of the beads in one example Elecsys® assay including signal intensity/signal dynamic.

If the sample batches meet the technical specifications both before and after conjugation, the remaining beads of the corresponding batch (or part of it) are accepted for conjugation. The conjugated beads are delivered as a concentrated suspension to Mannheim where the final Elecsys® assays are “assembled”.

For an Elecsys® kit, a so-called rack pack is put together with three “cartridges”: one containing the beads (suspended in Mannheim with a buffer); and two others containing two antibodies and further ingredients necessary for the assay. These are also produced as concentrates in Penzberg and then diluted in Mannheim. The quality control and standardization<sup>9</sup> of the assay are performed in Mannheim.

#### **b) Challenges to meet the requirements in the standard production process**

Based on LT’s extensive experience in bead production, reproducibility among batches of **coated beads** is challenging due to the very narrow parameters of the beads.

The **two most critical parameters are bead size and specific surface area**. For example, even a slight deviation in the surface area can result in decreased performance of the beads, and hence not meeting the technical requirements. The technical requirements of the Elecsys® assays are so demanding that even those beads meeting specifications may not perform adequately in the assays.

In addition, even if the specifications for the coated beads as tested by LT are met, in some circumstances **the conjugated beads** do not meet the required parameters, especially in the area of **biotin binding capacity and signal dynamics, which are critical parameters for the conjugated beads**.

Any changes in the production process (such as using an alternative solvent or changes in any other parameter) will thus be challenging. **If the beads do not provide a signal in the final assay that can be standardized for the ca. 33,000 Elecsys® instruments that are currently placed at customers’ worldwide, the beads cannot be employed in the assays.**

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<sup>9</sup> Standardization means determining the conversion factor, which converts the measurement values of this particular rack pack to the standard calibration that is given in the instrument. This information is saved in form of a barcode on the assay and read-out by the instrument. Thus the conversion of the signal is done automatically by the instrument.



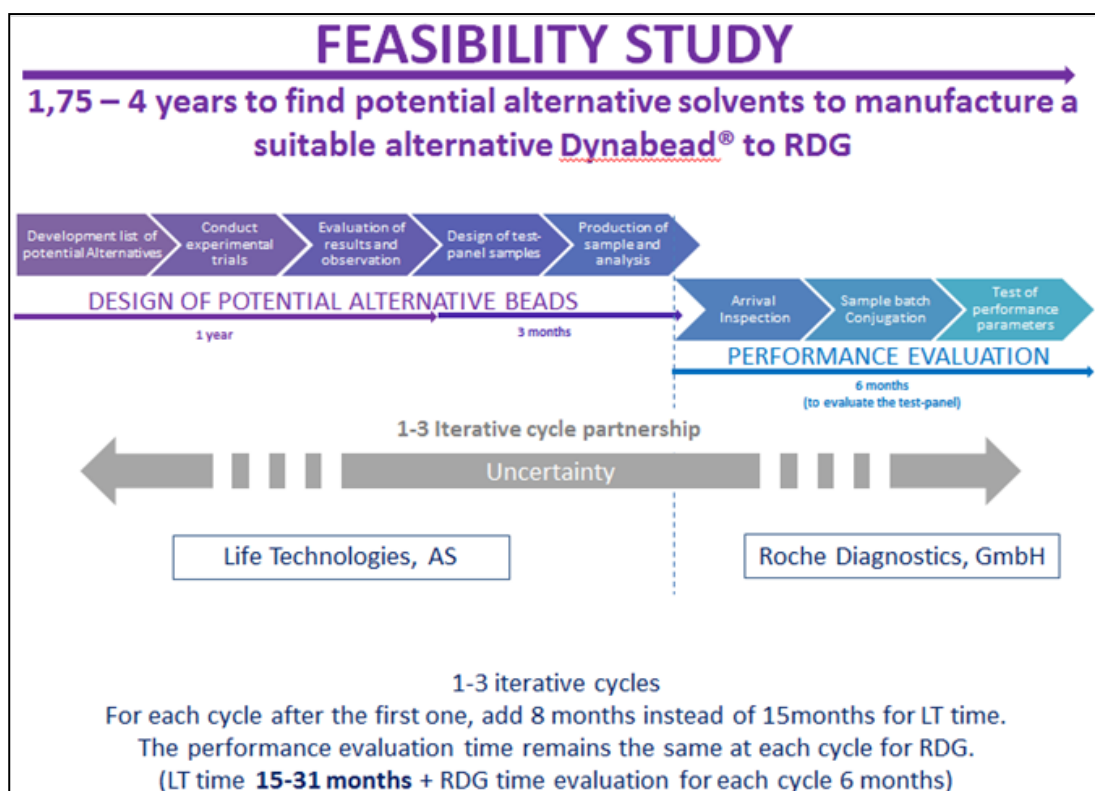
**5.2.2. Feasibility Study: Test Panel Performance Evaluation**

Test panel beads produced by LT using alternative solvents for the purpose of identifying substitutes for diglyme will first be tested in the R&D department rather than the production facility due to only small amounts being provided (feasibility study). The R&D department in Penzberg will test the conjugation of the beads, and also the performance of the Elecsys® assays (with one or several selected assays). This process is an iterative process with close collaboration between LT and RDG (Figure 3).

**Table 4: Overview of the different steps necessary to evaluate alternative beads, including time required for the 14 beads**

Steps in the Testing of Alternative beads	Time Required (test panel)
Standard procedure for coated beads (arrival inspection)	2 days
Conjugation of beads (small scale)	ca. 1 week
Standard procedure for testing of conjugated beads HBQ (=Hot-Bead-Quench-Test) to determine the background signal (noise) and CPS (CPS = disc centrifuge) to determine particle size distribution / aggregation	ca. 8 weeks (4 full time employees) or 16 weeks (2 full time employees)

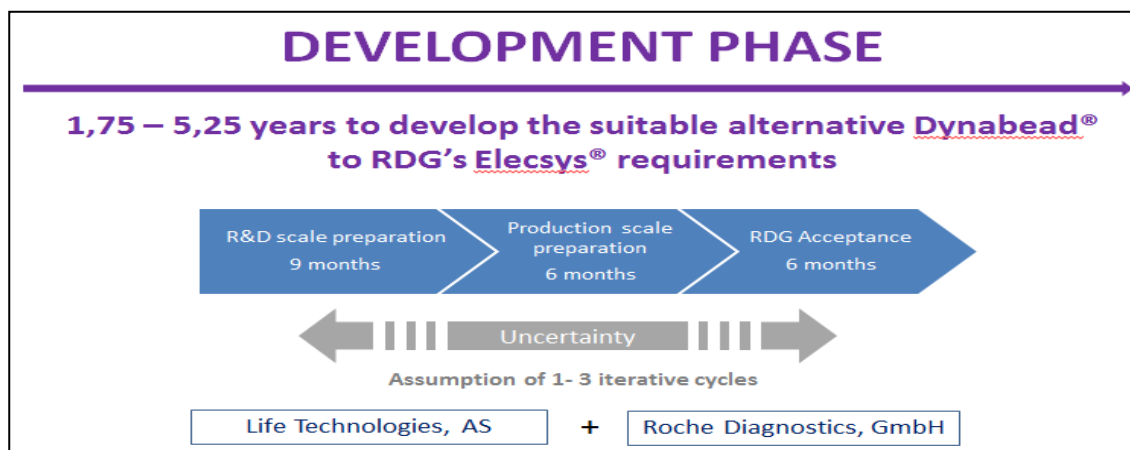
**Figure 3: Overview of the feasibility study**



### 5.2.3. Development Phase

Once the feasibility study is completed and a viable alternative solvent(s) is identified then a development phase to optimize the process is established (mainly taking place at LT) (Figure 4).

**Figure 4: Development phase**



### 5.2.4. Validation Phase and Market Authorisation

The validation step, performed after the process development by LT, consists of the verification of the beads (using alternative solvent(s)) in the Elecsys® assays. The validation activities would be performed based on the existing quality procedures in cooperation with R&D and Operations. Three lots would be needed and equivalency/validation studies with a group of assays (to be defined, including the highly regulated assays) need to be performed. Subsequently, a change notification for existing market authorisations would likely be needed. Table 5 gives an overview of the necessary steps to be performed by RDG. The timelines given below are based on the assumption that a change in solvent will only lead to a small change in the beads and the conjugation process remains the same. In the case that only the solvent in the production process is changed and the equivalency studies demonstrate similar performance to current beads (non-conjugated and conjugated) then no or minor further regulatory approvals or notifications are needed.

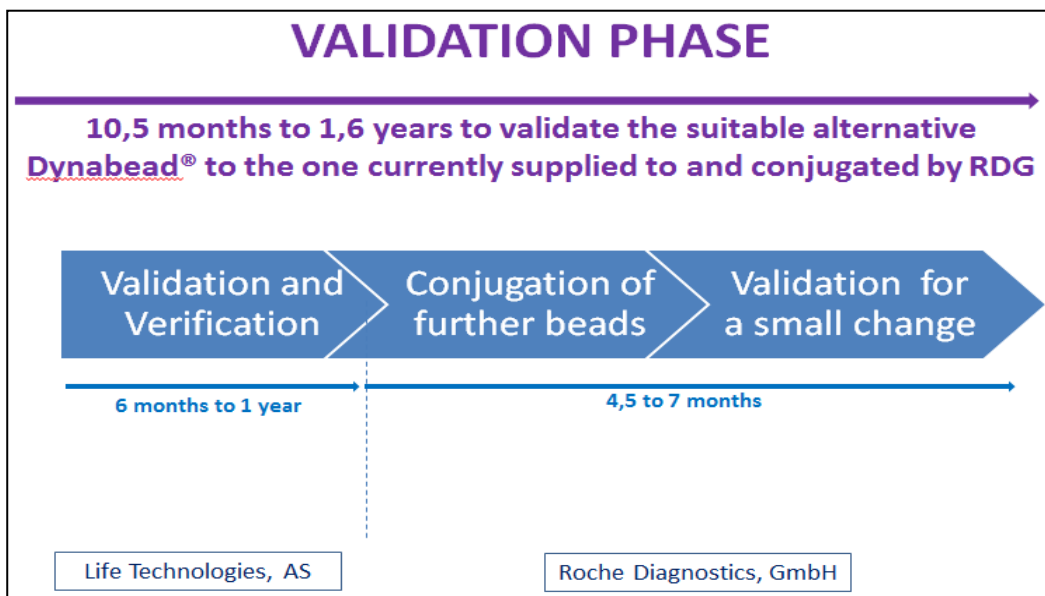
**Table 5: Validation and market authorisation in case of small changes – changes to the bead that do not require a change in the conjugation process**

Steps in the Validation of Alternative Beads	Time Required (one type of alternative bead)
If bead suitable: Conjugation of further beads for validation of the assays – 3 production batches	8 weeks (including manufacturing and QC)
Validation in case of a small change of the beads: 20 assays*	Minimum of 10 weeks (4 full time employees) 20 weeks (2 full time employees)
Market authorization: Change notification	10 weeks for the highly regulated assays (EU, U.S., China, Canada, other regions)
Authority evaluation of change notification	6 months to one year (except China, may take longer)

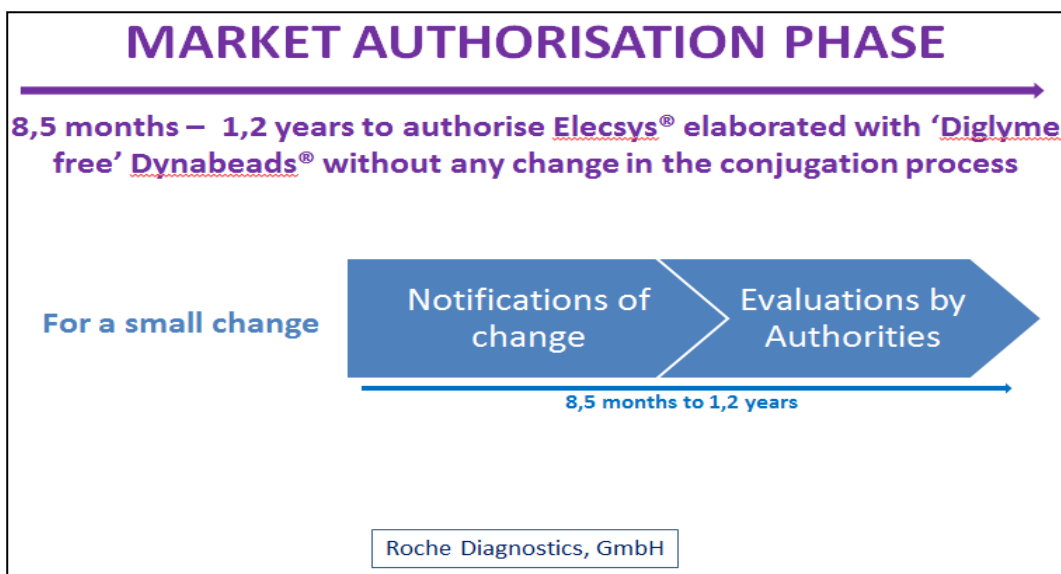
\* The minimum time required for validation of one Elecsys® assay is ca. 2 weeks (100 % of a full time employee) in case of small changes and similar performance

Figures 5 and 6 provide an overview of all steps to be performed by LT and RDG.

**Figure 5: Validation phase in case of a small change**



**Figure 6: Market authorisation in case of small change**



At the moment, there is no plan to change the technology of bead manufacturing (*i.e.*, changes to the functional group, coating or conjugation). Any such change would lead to the timelines presented in Table 6 (large changes) and this is considered not feasible as then new IVD regulatory submissions for allowing the Elecsys® assays on the market are needed. New market authorisation can take more than 20 years depending on the country/region and the respective regulatory requirements.

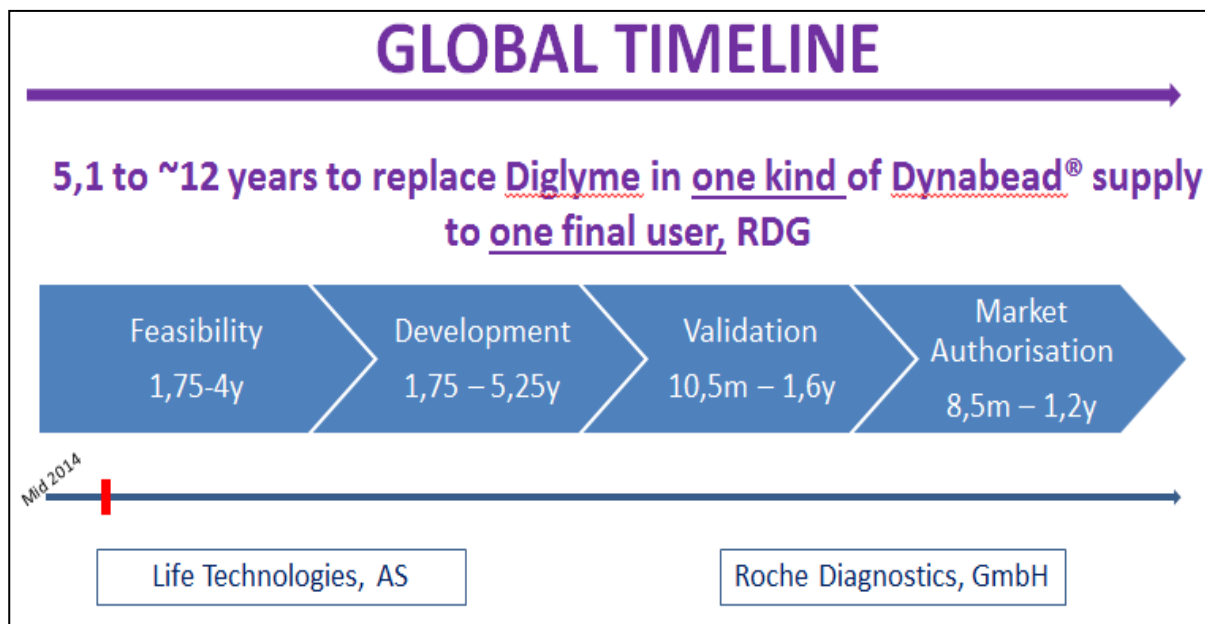
**Table 6: Validation and market authorisation in case of larger changes – changes that would require an adaptation of the conjugation process**

Steps in the Validation of Alternative Bead	Time Required (one type of alternative bead)
Redevelopment of one type of bead	1-3 years (hypothetical depending on type of change e.g. conjugation process change ...)
New bead or Conjugation process change 3 production batches	8 weeks
Validation in case of <b>larger changes</b> of the beads : all ca. 100 assays	Min. 1-2 years (6 or 3 full time employees)
Clinical studies for the regulatory relevant parameters. Assuming that this is needed for 30-50 parameters	Estimate 5-10 years assuming that clinical studies can be run on 6 assays in parallel per year. The costs involved amount to around 150-250 million dollars
New submission	Submissions globally per region. Examples: - US (PMA): 60 months for 10 highly regulated parameters - EU (15 IIA parameters): 15 months - China (50 assays): 150 months
Authority evaluation	1 to 3 years for EU and U.S. as an average (background: US (PMA): 6-9 months per assay EU submission: 4-6 months per assay China: 7-12 months per assay)

### 5.3. Overview of Timeline to Find an Alternative

Figure 7 summarizes the estimated global timeline necessary to find an alternative solvent for diglyme including market authorisation for the Elecsys® assays. The timeline given below is based on the assumption that a change in solvent will only lead to a small change in the beads and the conjugation process remains the same. If an alternative showing a satisfactory overall technical and functional performance is identified and validated, the production in Penzberg will be adapted based on the changes implemented at LT in Norway.

Figure 7: Global timeline (LT and RDG)



## 6. FURTHER R&D ACTIVITIES

At the time of submitting this application for authorization, **RDG did not have any experience in the production of Dynabeads® magnetic beads, and did not have the opportunity to do any research into alternative solvents for this specific process.**

RDG, as part of Roche worldwide, has a public commitment<sup>10</sup> to **substitute any Substances of Very High Concern (SVHC) used in its products or processes within 10 years after listing as SVHC (when technically possible).** Testing and validation work is on-going on alternative aprotic solvents (*e.g.*, a cellulose extract), but so far the results do not meet the requirements of the specific applications as compared to the traditional aprotic solvents (*e.g.* too high boiling point, solubility potential, viscosity too high...). Roche is also an active member of the ACS Green Chemistry Institute Pharmaceutical Roundtable<sup>11</sup> which encourages innovation while catalyzing the integration of green chemistry and green engineering into the pharmaceutical industry. In parallel, it has its own internal Green Chemistry Group which aims to make Roche processes safer and find less hazardous alternative chemicals to use throughout Roche. In 2010, RDG also issued an internal Roche/Genentech Solvent Selection Guide, which aims to deselect problematic solvents. In the selection guide, diglyme is listed in the "banned" category and is not used by RDG in any other process.

In case of failure to identify a suitable alternative solvent, **RDG would join LT's efforts to re-evaluate the solvent ranking list generated by the initial strategy and select additional solvents or mixture of solvents to test.** Such work can only start after the production process is established in Penzberg and experience has been gained. Therefore, such work would not start before 2018.

<sup>10</sup> See [http://www.roche.com/sustainability/for\\_communities\\_and\\_environment/environment/our\\_she\\_goals\\_and\\_performance.htm](http://www.roche.com/sustainability/for_communities_and_environment/environment/our_she_goals_and_performance.htm).

<sup>11</sup> Joint effort of the American Chemical Society, the Green Chemistry Institute® and a number of global pharmaceutical corporations.

## 7. CONCLUSION

To ensure the continuity of the supply chain and to meet the increasing market demand for the Elecsys® assays, RDG invested in the technology transfer of one specific type of Dynabeads®.

RDG is applying for an authorization to use diglyme in the planned future production of Dynabeads® in Penzberg.

For the time being **RDG does not have any experience in the production of Dynabeads® magnetic beads, and therefore has not had the opportunity to do any research into alternative solvents for this specific process.**

However, over the past 20 years, RDG has been depending on Dynabeads® supplied from LT as a core part in the functioning of the Elecsys® immunoassays. This magnetic bead plays an important role in the quantification of the target health marker in patient samples such as blood.

Over this time, RDG has built an excellent relationship with its unique supplier and has invested much effort in optimizing its assays with the existing process/specifications. Therefore, **an exchange of diglyme can only be possible if RDG can demonstrate that an exchange does not affect its assay performance.** RDG will be taking part in the research of an alternative to diglyme, by conducting performance evaluation of Dynabeads® manufactured with potential alternative solvents.

RDG is applying for 12 years because, as detailed in this AoA, for the foreseeable future there is no alternative solvent to diglyme. In case a technically viable substitute is identified, the development of alternative beads and the related revalidation process will require several years given the high sensitivity of the products. Finally, an additional time period should be taken into account in the event a new marketing authorization for Elecsys® assays would be required. Against this background, RDG has come to the conclusion that any review period shorter than 12 years would not be sufficiently long for identifying a viable substitute and completing the transition to a diglyme free process.

**Given the complexity, the sensitivity of the product, the stringent requirements for their unchanged performance, and the fact that an extensive validation phase cannot be dismissed, a long time frame (12 years) for the possible and safe exchange of diglyme is needed.**