Unit: Technical Assessment Unit

Public assessment report for biological products

IMMUNORHO, 300mcg /2ml

Administrative information:

Trade name of the medicinal product:	Immunorho, 300mcg /2ml
INN (or common name) of the active substance(s):	Human Anti-D Immunoglobulin
Manufacturer of the finished product	Kedrion S.P.A., S.S 7 Bis Km.19.5,80029 Sant Antimo,Napoli - ITALY
Marketing Authorization holder	Kedrion S.P.A LOCALITA AL CONTI - FRAZIONE DI CASTELVECCHIO PASCOLI 55051-BARGA (LU) - ITALY
Applied Indication(s):	- Prevention of Rh(D) immunisation in Rh(D) negative childbearing age women. - Treatment of Rh(D) negative childbearing age women after incompatible transfusions of Rh(D) positive blood or other products containing red blood cells e.g. platelet concentrate.
Pharmaceutical form(s) and strength(s):	- 300mcg /2ml Solution for injection in Pre-filled Syringe
Route of administration	I.M
Type of registration (EMA/FDA – Local)	Imported

List of abbreviations

APH	Ante-partum haemorrhage
AE	Adverse Event
EMA	European Medicines Agency
FDA	Food and Drug Administration
IM	Intramuscular
IV	Intravenous
IU	International unit

Ig	immunoglobulin
IUFD	intrauterine foetal death
ICH	International council of harmonization
IM	Intramuscular
RBCs	Red Blood Cells
Rh	Rhesus factor
TnBP	tri-n-butyl phosphate
TPH	Trans-placental haemorrhage
Tmax	Time to peak serum concentration
µg	microgram
RBCs	Red Blood Cells
Rh	Rhesus
Rh(D)	Inherited Protein of the Surface of Red Blood Cells that Bears the D Antigen

Table of contents

1. General introduction about the product including brief description of the AI, its mode of action and indications.

IMMUNORHO is a medicinal product belonging to the pharmacological class of immune sera and human specific immunoglobulins for intramuscular administration. It contains specific antibodies against erythrocytes D-antigens corresponding to 150mcg (750 I.U.) in each ml of finished product. It complies with the monograph *Human Anti-D Immunoglobulin* (0557) and with the monograph *Human Normal Immunoglobulin* (0338)

Immunorho is indicated for:
Prevention of Rh (D) immunization in Rh (D) negative women
 Antenatal prophylaxis
 Planned antenatal prophylaxis

 Antenatal prophylaxis following complications of pregnancy including: Abortion/threatened abortion, ectopic pregnancy or hydatidiform mole, intrauterine fetal death (IUFD), transplacental haemorrhage (TPH)

Postnatal prophylaxis
 Delivery of a Rh (D) positive (D, D weak, D partial) baby
Treatment of Rh (D) negative persons after incompatible transfusion of Rh (D) positive blood or other products containing red blood cells e.g. platelet concentrate.

2. Quality aspects:

2.2.1 Introduction

As mentioned in aforementioned section

2.2.2 Drug Substance (Active ingredient)

- **General information**

European Pharmacopoeia name: Human Anti-D Immunoglobulin

The Human Anti-D Immunoglobulin is a natural product derived from plasma of selected and immunized donors: the structure is defined by the fractionation method of manufacture and by physico-chemical characterization. Human Anti-D Immunoglobulin is a liquid preparation containing immunoglobulins, mainly immunoglobulins G. It contains specific antibodies against erythrocytes D-antigen. Human anti-D Immunoglobulin is obtained from plasma from selected and immunized donors having antibodies against erythrocyte D-antigen.

- **Manufacturer(s)**

The Drug substance is manufactured at two production sites (Kedrion S.p.A. Bolognana and Kedrion S.p.A. S.Antimo - ITALY. The site complies with the GMP requirements.

Description of Manufacturing Process and Process Controls.

Human anti-D immunoglobulin is manufactured through cold fractionation method which is based on the separation and precipitation of a specific protein species from other components of plasma.In this perspective the entire fractionation method should be regarded as a purification process intended to separate a specific protein species by recovering all other plasma components.

During the manufacturing process the following steps are documented as capable of reducing viral charge:

- Removal of Fraction I;
- Removal of Fraction II+III;
- Removal of Fraction III;
- Solvent/detergent treatment

Starting materials quantities could change according to Fraction II protein content.

- Three main phases, subdivided in different steps, can be identified in the manufacturing process of Anti-D Immunoglobulin Bulk solution:

1- Plasma thawing and plasma pool preparation

2- Cold ethanol fractionation

3- Immunoglobulin bulk preparation

A flow sheet diagram providing a complete visual representation of the manufacturing process from plasma to final Bulk, is reported in MA file

the flow diagram of each phase and the narrative description of the main operative conditions for each step, including process parameters such as temperature, pH, time, pressure, flow rates. This provides a clear understanding of each step of the manufacturing process, operations, conditions, operative ranges and controls applied. The flow sheet diagram identifies the steps at which samples are taken to check quality and reproducibility of operations and products.

The whole process is carried out within an overall quality management system that includes control of starting materials, raw materials, source of supply and relevant documents, routine in-process testing and manufacturing procedures.

Control of Materials.

Plasma pools are tested by Kedrion for HBV DNA, HIV-1 RNA and HAV RNA NAT in accordance with monograph 0853 *"Human Plasma for Fractionation"* of the European Pharmacopoeia; plasma pools for the manufacturing of anti-D immunoglobulin are also tested for B19 virus DNA, in accordance with monograph 0557 "Human *Anti-D Immunoglobulin".*

Detailed information on the validation of testing methods and mini-pool strategies are reported in the current valid Plasma Master File (PMF/EMEA/H/000012/07/IB/002, filed separately) which includes all the data required by the relevant current EU guidelines.

The materials used throughout the manufacturing process up to Anti-D Fraction II, and Immunoglobulin Bulk solution starting from Fraction II are received with a Certificate of analysis by the supplier and are listed in tables.

Controls of Critical Steps and Intermediates.
In process critical process steps parameters are mentioned in the manufacturing process flow chart Anti-D Fraction II paste represents the intermediate in the manufacturing process of immunorho.

-The intermediate Fraction II, used as starting material is manufactured and controlled by Kedrion Bolognana plant. -Fraction II derives from Fraction II+III. This is a suspension that is currently processed either immediately or within a few days of manufacturing: in this case it is stored at –20°C or below. The intermediate anti-D Fraction II paste, is controlled according to the following specifications: Bacterial endotoxin, Protein composition and Anti-D Antibodies

Process Validation
The process validations carried out confirmed that the Immunrho production process is well controlled and consistently and reproducibly delivers a drug product meeting its acceptance criteria and product quality attributes

-

Manufacturing Process Development.

Human anti-D Immunoglobulin for intramuscular use is manufactured through cold fractionation method. The manufacturing process is sufficiently describing the whole changes made to the DS manufacturing process with proper justification. Detailed description for each step development is mentioned in the MA file.

Characterization.

the structure is defined by the fractionation method of manufacture and further confirmed by compliance with European Pharmacopoeia identification requirements and physico-chemical characteristics.

Potential impurities deriving from manufacturing of immunoglobulin preparation are according to European Pharmacopoeia.

Specification:

Specification limits for anti-D immunoglobulin Bulk solution have been derived by the reproducibility of results of a consolidated industrial process.

The tests performed on the drug substance comply with the acceptance criteria of the European Pharmacopeia.

Analytical Procedures.: analytical procedures with validation reports are provided in the CTD file

Batch analysis: -The results of drug substance compared to the data obtained from the consistency batches are provided in the CTD file and found to be satisfactory

Reference Standards or Materials.:

List of Reference standards or materials used for test analysis of bulk is provided.

Container closure system:
Bulk solution produced is formulated and then collected in ***plastic bags*** before being sterile filtered and filled into the final containers.

- **Stability of drug substance:** Based on available stability data

Approved shelf life:

- 1- Intermediate (Fraction II): 2 years
- 2- Active substance: Maximum 2 years

Approved Storage Conditions:

- Intermediate (Fraction II): Store at temperature ≤ -20 °C
- Active substance: Store at temperature between +2°C - +8°C

2.2.3 Drug product:

- **Description and Composition of the Drug Product:**

- IMMUNORHO is a virus inactivated human anti-D immunoglobulin for intramuscular administration.

- Pharmaceutical Development including brief description on Components of drug product.
IMMUNORHO 300 mcg/2 ml solution for injection. Each 1ml of solution contain Anti D-Immunoglobulin Bulk solution is conventionally taken as the drug substance with excipients sodium chloride, Glycine and water for injection.

The product complies with the requirements of the monograph n° 0557 *"Human anti-D immunoglobulin"* reported in the Eur. Ph., current edition.

-The product is manufactured using human venous plasma, which meets the specifications of the monograph n° 0853 *"Human plasma for fractionation"* of the Eur Ph. current edition

- Formulation Development
- **Overages:** No overages are applied. An overfill, expressed as average per cent value

- Physicochemical and Biological Properties
The physicochemical and biological properties of Immunorho were investigated.

- **Manufacturing Process Development:** The steps from the Bulk solution to the finished product comprises a sterile filtration followed by filling of the Bulk solution into the final containers, carried out.

- Container closure system and their compatibility.
The sterile anti-D immunoglobulins bulk solution is aseptically filled in pre-filled syringes (2ml). Stoppers are controlled to ascertain the compliance with current Ph. Eur. current ed., 3.*2.9. "Rubber closures for containers for aqueous parenteral preparations, for powders and for freeze dried powders".*

The available stability data related to IMMUNORO 300 mcg solution for injection show no significant changes.

- Microbiological Attributes.:
The filled product is sterile.
The culture medium in the syringes doesn't show microbial growth of test microorganism and the data has been reviewed and found to be satisfactory.

- Compatibility.
No interaction due to adsorption or degradation and no change in pH values have been showed during the checked period.

- **Manufacture of the drug product:**

The finished product is manufactured at: KEDRION S.p.A. S.S. 7 bis Km. 19,5 - 80029, Sant'Antimo (NA), Italy. Manufacturing of DP is performed in accordance with cGMP regulations

- **Description of manufacturing process and process controls along with manufacturers and responsibilities**.
- A flow diagram is clearly presented giving the steps of the process and showing where materials enter the process. The critical steps the process control parameters and points at which process control

or final product controls are conducted are identified. A narrative description of the manufacturing process, including filtration, filling of immunoglobulin bulk solution in the final container and stoppering, labelling and packaging are provided.

Control of critical steps and intermediates:
Sterile filtration and filling of immunoglobulin bulk solution have been identified as critical operations during the preparation of IMMUNORHO starting from bulk solution.
Since the final bulk product represents the drug substance and the production process is a continuous process, no intermediates are defined.

- Process validation and / or evaluation.
Validation of the final filtration step and filling operations has been carried out and the data is supplied by the manufacturer, assessed technically & found to be satisfactory

Product specification:
IMMUNORHO is a liquid preparation containing immunoglobulins (mainly immunoglobulin IgG) obtained from human plasma that complies with the following control tests specifications according to Ph. Eur. current edition, monograph 557 “Human Anti-D immunoglobulin” and monograph 338 “Human Normal Immunoglobulin", and *ICH guidelines Q6B.*

-Glycine, Sodium chloride and Water for injections (WFI) are the excipients present in the final product formulation. -Glycine is used a stabilizer, Sodium Chloride is used to make the preparation isotonic and the WFI represents the medium solvent.
Specifications, control tests of all the excipients present in the final product formulation.
performed and limits follow the European Pharmacopoeia
Excipients of Human or Animal origin are not used for the production of Immunorho.
There is no novel excipients used.

Characterization of impurities.:
The types of potential impurities in the drug product are the same of those present in the drug substance. Additional tests are carried out with the aim of verifying that levels of residues of raw materials used in the manufacturing process comply with predetermined specifications adequate for the use of the product.
-Manufacturing residue levels have shown no safety problems over a long-term clinical use.

Batch analysis
Data are provided for 3 batches of the finished products in form of respective Certificates of Analysis and all results comply with specification.

Reference Standards or Materials.:

Complete and detailed information on the reference standards and reference materials used for testing the drug product is provided in the relevant standard operating procedures

Container closure system.: container closure system components comply with the requirements of the European Pharmacopoeia and the United States Pharmacopoeia.

- **Stability of the drug product.**
  Approved shelf life for the Finished product:
  Approved Shelf Life for finished Product: 3 years
  Finished Product:
  Store in a refrigerator (2°C - 8°C) and do not freeze.
  Keep in the original container and in the outer carton in order to protect from light

3. Non –clinical aspect:

➢ **Immunorho** is a human anti D immunoglobulin product containing specific neutralizing antibodies (mainly IgG) against erythrocytes RH (D) obtained from plasma fractionation of healthy donors immunized against D-antigens, acting by suppressing the immune response in Rh negative individuals who are exposed to Rh D positive red cells, thus preventing the development of antibodies to Rh D. It is indicated for the prevention of Rh(D) immunisation in Rh(D) negative childbearing age women as antenatal or postnatal prophylaxis. It is also used to treat Rh(D) negative childbearing age women after incompatible transfusions of Rh(D) positive blood or other products containing red blood cells e.g. platelet concentrate. This product is not approved by EMA nor FDA, but was approved by the national regulatory authority in Italy on November 25th, 1982 as powder and solvent for solution for injection, and in 2010 as a pre-filled syringe.

➢ **Pharmacology:** No pharmacological studies related to the active substance were performed due to the nature of the product.

➢ **Pharmacokinetics:** No PK studies related to the active substance were performed due to the nature of the product.

➢ **Toxicology:** No toxicology studies related to the active substance were performed due to the nature of the product.

- The solvent/detergent step of manufacturing utilizes Tri-n-butyl-phosphate (TnBP) and Sodium Cholate; traces of the two components are still present in the finished product. The toxicological potential of these two contaminants was evaluated through a literature review.
- Generally, the ICH guideline Q3C(R6) on impurities considers TnBP as a low toxicological potential solvent. Notably, the TnBP is well characterized toxicologically, by oral, dermal and inhalation routes. Due to its high oral absorption, it can be considered that IV and oral animal toxic effects are similar even IV route offers lower exposure lower than after oral administration. Sodium cholate animal toxicity was not systematically investigated by parenteral route. No IM data have been reported, it is

however expected that the bioavailability of IV administration covers that of IM route. According to toxicological literature on TnBP and Sodium Cholate, these two contaminants do not pose any toxicological concern during the therapeutic use of immunorho.

- **Overall conclusion:** There are no preclinical concerns with this product since the level of two contaminants (TnBP & Sodium Cholate) is well below the limits where any toxicity may be expected.

4. Clinical aspect:

The clinical development program for Immunorho includes two Phase I studies (KB038bis and KB073) evaluating pharmacokinetics (PK), tolerability, and safety in healthy Rh(D)-negative volunteers (males and females), and one Phase III study (KB065) assessing efficacy, PK, and safety in Rh(D)-negative pregnant women carrying Rh(D)-positive fetuses.

Across all trials, study designs were open-label and non-controlled, consistent with the established pharmacological and ethical framework for anti-D immunoglobulin assessment. Immunorho was administered intramuscularly at a standard dose of 300 mcg (1,500 IU).

The primary clinical objectives were:

- Characterization of PK parameters following IM administration.
- Assessment of local/systemic safety and tolerability.
- Evaluation of prophylactic efficacy in preventing Rh(D) alloimmunization in pregnancy (Phase III).

The data consistently demonstrate predictable absorption and elimination kinetics, acceptable inter-subject variability, and a safety profile consistent with licensed anti-D immunoglobulin products. The Phase III study confirms the prophylactic efficacy of Immunorho with no observed cases of alloimmunization.

- **<u>Clinical Efficacy and Immunogenicity</u>**

  Efficacy was not assessed in the two Phase I studies (KB038bis, KB073), as expected for early-phase PK and safety evaluations.

Phase III Efficacy Findings (KB065):

- Population: 255 Rh(D)-negative pregnant women carrying Rh(D)-positive fetuses.
- Primary Endpoint: Incidence of anti-D alloimmunization at 24 weeks post-final dose.
- Result:
  - Zero cases of true alloimmunization were detected.
  - Positive anti-D findings by Coombs testing (7.0%) represented **passive immunization**, confirmed by declining titers (<2) and RBC alloantibody identification.

- No subjects developed clinically significant anti-D antibodies.

Secondary Endpoint (12-week assessment):

- High frequency of anti-D detection at Week 12 (81.6%); all cases confirmed as passive antibody presence post-injection.
- No cases of alloimmunization observed.

Immunogenicity Profile:

- Consistent, predictable development and gradual decline of passively acquired anti-D antibodies.
- Antibody profiles aligned with expected pharmacokinetic clearance, supporting appropriate duration of protection through pregnancy and postpartum periods.

Overall, Immunorho achieved complete prevention of Rh(D) alloimmunization in the studied population.

- **Clinical Safety**

Safety was evaluated across all three studies in 288 total exposed subjects.

Phase I Safety (KB038bis):

- Only four mild, non-drug-related AEs were reported (primarily headache).
- No injection-site reactions, no clinically relevant laboratory changes except minor reductions in hemoglobin/hematocrit related to phlebotomy.
- No vital sign abnormalities.
- Concluded as well tolerated.

Phase I Safety (KB073):

- 72.2% of subjects experienced TEAEs; all were mild or moderate.
- No serious AEs, no withdrawals due to AEs.
- Mild treatment-related local reactions (warmth, pruritus, injection-site reaction) occurred in a small number of subjects.
- No clinically significant ECG, laboratory, or vital sign abnormalities.
- Concluded as safe and well tolerated.

Phase III Safety (KB065):

- TEAEs reported in 60% of subjects, consistent with pregnancy-related conditions and background obstetric events.
- Most common TEAEs: procedural pain, anemia of pregnancy, headache.
- Drug-related TEAEs were infrequent (1.6%) and mild (somnolence, pruritus).
- No cases of serious unexpected adverse reactions attributable to the investigational product.
- Injection-site tolerability acceptable.

- No clinically significant abnormalities in laboratory parameters, vital signs, or obstetric outcomes attributable to Immunorho.

Safety findings across all studies conform to the known safety profile of licensed anti-D immunoglobulin preparations.

➢ **Overall Conclusion**

Across Phase I and Phase III studies, Immunorho demonstrated:

- **Reliable pharmacokinetics**: slow absorption (Tmax ~48 -74 hours in volunteers; ~7 days in pregnancy), prolonged elimination half-life (~23-24 days), and exposure consistent with maintaining protective anti-D levels.
- **Robust prophylactic efficacy**: No cases of Rh(D) alloimmunization occurred in the Phase III study. All detected anti-D antibodies represented expected passive immunization.
- **Favorable safety and tolerability**: No unexpected safety signals; majority of adverse events were mild and non-drug-related. Injection-site reactions were minimal. Safety outcomes align with established intramuscular anti-D immunoglobulin products.

The clinical evidence confirms that Immunorho is effective in preventing Rh(D) alloimmunization and is well tolerated in both healthy volunteers and pregnant patients.

➢ **Benefit–Risk Evaluation**

Benefits:

- Complete prevention of Rh(D) alloimmunization in the studied pregnant population.
- Predictable pharmacokinetic profile ensuring sustained passive antibody levels across the critical antepartum and postpartum periods.
- Established mechanism of action consistent with decades of clinical use of anti-D immunoglobulins.

Risks:

- Adverse events were mostly mild, transient, and consistent with known effects of immunoglobulin preparations and pregnancy.
- No serious treatment-related AEs, no injection-site complications of clinical concern, and no immunogenicity safety risks.

Benefit-Risk Conclusion:

The benefit-risk profile is favorable. Immunorho provides effective prophylaxis against Rh(D)

alloimmunization a condition associated with severe fetal and neonatal morbidity while demonstrating excellent tolerability and no safety concerns beyond the expected profile of anti-D immunoglobulins.

Therefore, Immunorho is supported as a safe and effective option for routine antenatal and postnatal prophylaxis in Rh(D)-negative women carrying Rh(D)-positive fetuses.

5. General Conclusion and Recommendations if any:

Based on the review of CTD modules and other supplementary documents, the product is approved.