DOSSIER FOR REGISTRATION OF

MF III of Switzerland HP

Laboratoires Dom-AVMM SUISSE
6340 Baar Schweiz-Suisse-Switzerland

1. 商品名

MF III of Switzerland HP

Each 2ml of MF III of Switzerland HP is derived from 230 g of fresh human Placenta

2. 服用方法 ：非経口

3. Active Substance(s)
Substance Name **Human Placenta Extract**

Strength of Substance 230 mg / 2ml

Each 2ml of MF III of Switzerland HP is derived from 0.230 g of fresh human Placenta
Total Nitrogen content NMT 0.08% w/v
窒素含有ＮＭＴ **0.08%w/v**

Pharmaceutical

SH: PRODUCT FORMULA

B1.1 Batch Manufacturing Formula

Batch Size: 300 Unit: J

B1.2 Attachment of Batch Manufacturing Formula Documentation
(Compulsory for imported product)

Sl. No	コード	原材料	原材料基準	ｍＬ当たりのラベル表示容量	2ｍＬ当たりの容量	240 リットル当たりの容量
ACTIVE INGREDIENT（活性原材料）						
1.	RRP28	Fresh human Placenta (as Extract) フレッシュヒトプラセンタ（抽出物として）	IH	0.230 g (Total Nitrogen content not more than 0.08% w/v 窒素容量 0.08% w/v を下回る)	0.230 g	27.6 kg
EXCIPIENTS						
2.	RRW03	注射水（注射のための水）	BP	q.s.（適量）	2 mL まで	240 まで

SECTION C: 梱包明細

LISTING OF PRODUCT PACKING

C1. Pack Size Please fill at least one of the following

Volume	:	2ml
Quantity	:	10 vials per tray x 2 trays or 10 vials per tray x 5 trays

C2. Immediate Container Type

Container	:	Glass vial bottles
Type Description	:	R2

C3. Barcode/Serial No: NA

SECTION D : LABEL (MOCKUP) FOR IMMEDIATE CONTAINER, OUTER CARTON AND PROPOSED PACKAGE INSERT

Please create digital Images (in JPEG,GIF,DOC,PPT format) by scanning hardcopy using scanners or snapshot images using digital camera.

D1 Label (mockup) for immediate container:

D2 Label (mockup) for Outer Carton:

SECTION E: SUPPLEMENTARY DOCUMENTATION

E1.1 Licensed Owner*:* **Laboratoires Dom-AVMM SUISSE**
6340 Baar Schweiz-Suisse-Switzerland

MF III OF SWITZERLAND HP

配合 ：
Each ml of MF III of Switzerland HP is derived from :
0.165 g of fresh Human Placentae.
Total Nitrogen : Not more than 0.08% w/v.
Water for Injection BP q.s.

Indications :
Wound healing processes and in conditions of delayed cicatrisation, burns, atonic ulcers, varicose ulcers, decubitus ulcers, fistulae, bronchial, oesophageal, gastric, cervical erosions associated with inflammation.
Various painful inflammatory processes associated with – Ulcers, Fractures, Erosions, Burns, Wounds, Pelvic inflammatory disorders.
In all immunodeficient states associated with chronic illness and debilitated conditions.

Tolerability : The drug has excellent tolerability. LD 50 is unnoticeable even in maximum doses that can be given.

Teratogenicity : None
The drug does not have any antigenic power.

Contraindications :
Individual hypersensitivity. No contraindications unlike NSAIDS which are contra-indicated in Peptic Ulcer disease, moderate / severe Hepatic impairment, Pregnancy & Lactation.

Undesired side effects :
The natural origin of the components allow absorption without side effects.

Drug interaction :
Not known.

用法・容量 ：(筋肉注射のみ)
通常２ｍｌ注射を毎日、あるいは1日おきに注射し、14回まで到達すれば十分。

Storage : Store in a cool dark place.

HIV ANTIBOY FREE, HEPATITIS B & C SURFACE ANTIGEN FREE

Presentation : 2 ml in glass vial bottles

Manufactured by
Laboratoires Dom-AVMM SUISSE
6340 Baar Schweiz-Suisse-Switzerland

E15. Manufacturer :

Laboratoires Dom-AVMM SUISSE
6340 Baar Schweiz-Suisse-Switzerland

E16. Other manufacturer(s) Involved (If any):

None

Processing Type: Please Select Assembly Production Repacker Labeling Others
GMP Certificate Attachment:
(Notes : GMP Certificate or Manufacturer licence is compulsory for overseas manufacturer)

E17. Store Address (If any):

None.

PART-II : QUALITY

SECTION (F)

S. Drug Substance For MF III OF SWITZERLAND HP INJECTION

S1. GENERAL INFORMATION

S1.1 Nomenclature	:	NA
S1.2 Structure	:	NA
S1.2.1. Attachment For Structure	:	NA
S1.3 General Properties	:	NA

S2. MANUFACTURER

S2.1. Manufacture Name	:	M/S
S2.1.1 Remarks	:	NA

S2.2. Description of manufacturing Process and Process Controls:

Annexure IV

S2.3. Controls of Materials:

Annexure V

S2.4. Controls of Critical Steps and Intermediates:

Annexure VI

S2.5. Process Validation and/or Evaluation:

The active ingredient of MF III of Switzerland HP Injection is the aqueous extract of human placenta. The placenta is a natural human origin and complex biological nature. Careful selection of source materials is the most important criterion for the efficacy and safety of medicinal product. Some selection tests are performed on collected human placenta to detect or qualify either antigens or antibodies. The human placenta is a very complex nature and it is difficult to interpret which is necessary to take into consideration for process validation. Further research is needed to develop an understanding of the most appropriate methodology for validation studies. Therefore, validation studies are currently not generally required.

S2.6. Manufacturing Process Development:

The Drug substance, Placenta, is a natural human origin. Therefore, Manufacturing Process development is not applicable.

S3. CHARACTERISATION

S3.1 Elucidation of structure and characteristics: NA

S3.2 Impurities : NONE

S4. CONTROL OF DRUG SUBSTANCE

S4.1 Specification:

Annexure VII , attached

S4.2 Analytical procedures:

Annexure VIII , attached

S4.3 Validation of Analytical procedures:

VALIDATION OF ANALYTICAL PROCEDURES FOR PLACENTA EXTRACT SOLUTION DERIVED FROM HUMAN PLACENTA

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. Typical analytical performance characteristics that should be considered in the validation of the types of methods described in the document are Accuracy, Precision, Specificity, Detection Limit, Quantitation Limit, Linearity and Range. Revalidation of analytical procedures may be necessary in the following circumstances:

changes in the synthesis of the drug substances; changes in the composition of the drug product; and changes in the analytical procedure.

For validation of analytical procedures of Placenta Extract Solution five lots were included in these studies and were identified namely by C00110, C00111, C00112, C00113 and C00114.

The following Specifications and Methods of Analysis for Placenta Solution were used for the validation studies.

Validation of in-house methods of analysis for placenta Extract Solution.

Description : (Limit- Colurless to pale yellow coloured clear liquid)

Results : All five lots of Placenta Extract Solution met the monograph specification.

Discussion : No problems were encountered in conduction this test.

Identification : (Limit- Chromatographic analysis of amino acid profile should match with the standard HPLC chromatogram)

Results : All five lots of Placenta Extract Solution met the monograph specification.

Discussion : The test present no analytical problems.
pH : (Limit- 6.00 to 7.50)

Results :

Placenta Extract Solution	Test-1	Test-2	Test-3
Lot C00110	pH-6.84	pH-6.85	pH-6.85
Lot C00111	pH-6.93	pH-6.91	pH-6.93
Lot C00112	pH-7.03	pH-7.01	pH-7.02
Lot C00113	pH-6.91	pH-6.92	pH-6.92
Lot C00114	pH-6.96	pH-6.96	pH-6.97

Discussion : All five lots of Placenta Extract Solution conformed to the monograph limits. The test present no analytical problems.

Heavy Metals : (Limit-Not more than 10 ppm).

Results : All five lots of Placenta Extract Solution met the monograph limits.

Discussion : There were no problems associated with this test.

Arsenic : (Limit-Not more than 1 ppm).

Results : All five lots of Placenta Extract Solution met the monograph limits.

Discussion : No problems were encountered in conducting this test.

Sulphated Ash : (Limit- Not more than 0.1% w/v).

Results :	Placenta Extract Solution	Test-1	Test-2	Test-3
	Lot C00110	0.070%	0.076%	0.074%
	Lot C00111	0.073%	0.075%	0.076%
	Lot C00112	0.072%	0.072%	0.073%
	Lot C00113	0.073%	0.073%	0.077%
	Lot C00114	0.072%	0.076%	0.073%

Discussion : There were no problems associated with this test.

HIV Antibody : (Limit- HIV Antibody must be absent)

Results :	Placenta Extract Solution	Test-1	Test-2	Test-3
	Lot C00110	Absent	Absent	Absent
	Lot C00111	Absent	Absent	Absent
	Lot C00112	Absent	Absent	Absent
	Lot C00113	Absent	Absent	Absent
	Lot C00114	Absent	Absent	Absent

Discussion : All five lots of Placenta Extract Solution met the specified limits. There were no problems associated with this test.

Hepatitis B Surface Antigen : (Limit-Hepatitis B Surface Antigen must be absent).

Results :	Placenta Extract Solution	Test-1	Test-2	Test-3
	Lot C00110	Absent	Absent	Absent
	Lot C00111	Absent	Absent	Absent
	Lot C00112	Absent	Absent	Absent
	Lot C00113	Absent	Absent	Absent
	Lot C00114	Absent	Absent	Absent

Discussion : All five lots of Placenta Extract Solution met the requirements of the monograph. No problems were encountered in conducting this test.

HCV Antibody : (Limit-HCV Antibody must be absent)

Results :	Placenta Extract Solution	Test-1	Test-2	Test-3
	Lot C00110	Absent	Absent	Absent
	Lot C00111	Absent	Absent	Absent
	Lot C00112	Absent	Absent	Absent
	Lot C00113	Absent	Absent	Absent
	Lot C00114	Absent	Absent	Absent

Discussion : All five lots of Placenta Extract Solution met the requirements of the monograph. There were no problems associated with this test

Assay for Total Nitrogen : (Limit- Total Nitrogen is not more than 0.08%w/v)

Results : Placenta Extract Solution

	Lot C00110	Lot C00111	Lot C00112	Lot C00113	Lot C00114
A)	0.0148%	0.0142%	0.0150%	0.0146%	0.0146%
B)	0.0151%	0.0142%	0.0146%	0.0145%	0.0138%
C)	0.0143%	0.0137%	0.0155%	0.0152%	0.0144%
D)	0.0151%	0.0134%	0.0153%	0.0142%	0.0147%
E)	0.0146%	0.0146%	0.0147%	0.0143%	0.0137%
Average	0.01478%	0.01402%	0.01502%	0.01456%	0.01424%
S.D.	0.000316	0.000316	0.000316	0.000316	0.000316
Relative S.D.	2.1380%	2.2553%	2.1052%	2.1717%	2.2205%
Range	0.0143% to 0.0151%	0.0134% to 0.0146%	0.0146% to 0.0155%	0.0142% to 0.0152%	0.0137% to 0.0146%

Discussion : In the assay for total nitrogen the average assay values, standard deviations, relative standard deviations and ranges produced were quite satisfactory. All five lots of Placenta Extract Solution met the monograph limits. No problems were encountered in conducting this test.

S4.4. Batch Analysis (Minimum 2 batches):

Annexure IX, attached

S4.5. Justification of Specification:

The specification of the finished product is designed to identify, characterize and ensure presence of specified constituents in the product.

S5. REFERENCE STANDARDS OR MATERIALS INFORMATIONS:

NA

S6. CONTAINER CLOSURE SYSTEM:

SS 316 autoclaveable , closed containers

S7. STABILITY DATA (Not Applicable) (Minimum 2 batches):

NA

PART-II : QUALITY

SECTION (G)

P. DRUG PRODUCT

P1. Description and Composition:

Colourless to pale yellow coloured clear liquid.

Each ml of MF III of Switzerland HP is derived from 0.165 g of fresh human Placentae
Total Nitrogen content NMT 0.08% w/v

P2. PHARMACEUTICAL DEVELOPMENT

P2.1. Information on Development Studies:

The truly transnational, biochemical and pharmacological studies coupled with cogent clinical evaluations of several years confirm outstanding therapeutic benefits offered by MF III OF SWITZERLAND HP in the management of wound healing, depressed immunological conditions and stubborn inflammatory disorders.

MF III OF SWITZERLAND HP is an original research product of our laboratory involving highly sophisticated processes of extraction from the biochemically enriched fresh healthy human placenta. MF III OF SWITZERLAND HP is made to stringent quality control standards that match international specifications.

P2.2. Components of The Drug Product: MF III of Switzerland HP

P2.3. Finished Products: Each ampoules contains 2ml of 230 mg of human placenta

P2.4. Manufacturing Process Development :

The objective of the manufacturing process development stage is to begin to translate the laboratory developed formulation into one that can be manufactured on a large scale in secondary production. Thus the pharmaceutical scientist has to know where the medicine will be manufactured, the equipments available at those sits, any particular restrictions on the availability of the desired excipients and environmental restriction such as a discharge of solvents into the atmosphere etc. A scientifically perfect medicine that creates production difficulties is less desirable than a compromise that is scientifically sound but capable of manufacture under all expected conditions.

Consideration also has to be given to the regulatory status of the excipients; are they materials approved in the pharmacopoeia (an official book of standards) of each country, are they readily available at reasonable cost, their qualities (e.g. purity, moisture content, particle size, microbial content) equivalent to those of the material studied during formulation

development ?. Frequently pharmacopoeial standards relate only to chemical properties of the excipient while, for a robust product, consistent physical properties are often more important. If a particular particle size range of an excipient is required, steps have to be taken at this

stage to identify the limits that are acceptable and to set appropriate specifications that can be adhered to.

Similar considerations apply to packaging components. If the package contributes significantly to the stability of the medicine (e.g. as a barrier for maintenance of sterility for an injection) confirmation that suitable packaging materials are available at all manufacturing sites must be obtained.

Once suitable excipients have been chosen and tentative specifications set, experimental work continues on a gradually increasing scale to determine the processing parameters for each stage of the process.

The scale of the process development work relates directly to the likely batch size of the production process. Furthermore, as by this stage in the development of the medicine extensive clinical trials will be proceeding, manufacture of the clinical trial supplies will be performed on an increasing scale so providing valuable information on the process. The formulation development work would probably be undertaken on a 1-5 litre scale, gradually working up through 10-30 litre batches at the process development / clinical trial stage.
As unforeseen problems can occur during scaling up, for example non-equivalence of different size manufacturing vessels, differences in stirring conditions, temperature changes due to the longer time bulk quantities take to heat up / cool down that small quantities, the objective at the scale up stage is to proceed stepwise and only to commit large quantities of drug, still likely to be in limited supply prior to manufacture of production quantities, when reasonable confidence exists of a successful outcome.

The above example would be scaled up through 100 litre batches eventually to 300 litre batches as full production trails. Usually a minimum of three production trails will be undertaken with production staff learning about the process and probably taking sole charge of the final batch. These production trials may form the lunch stocks of the new medicine and therefore it is important that all steps are taken to guarantee the quantity of those batches through thorough adherence to the principles of Good Manufacturing Practice (GMP). These are regulations designed to assure the quality of products through in-built control in the production process. It is now accepted through in-built control in the production process. It is now accepted that one ‘builds’ quality into a product from the outset as described here rather than simply ‘Controlling’ quality by analysis of a limited sample after manufacture.
The successful outcome of the process development and scale-up stages is the marketed medicine. The formulation will have been soundly developed, the package secure, the process optimized for robustness, economy and quality, and if all goes well the pharmaceuticals scientist will never be called into secondary production as no problems will arise.

P2.5. Container Closure System : 2 ml glass vial bottles

P2.6. Microbiological Attributes : Sterile

P2.7. Compatibility : NA

P3. CONTRACTING MANUFACTURER : MV Dr. Daniel Elias – Vetchem Mevak a.s, European Union

P3.1 Batch Formula:

Sl. No	Code	Raw Materials	Raw Materials Standard	Label Claim per mL	Quantity / 2 mL	Quantity per 240 liters
ACTIVE INGREDIENT						
1.	RRP28	Fresh human Placenta (as extract)	IH	0.1 g (Total Nitrogen content not more than 0.08% w/v)	0.230 g	27.6 kg
EXCIPIENTS						
2.	RRW03	Water for Injection	BP	q.s.	to 2 mL	to 240 liters

P3.2 Manufacturing Process and Process Control:

Annexure X

P3.2.1 Manufacturing Process flowchart (if any):

Annexure XI

P3.3 Control Of Critical Steps and Intermediates: NA

P3.4 Process validation and/or Evaluation:

An important factor in the assurance of product quality includes the adequate design and control of the manufacturing process. Routine end product testing alone is not necessarily sufficient because of limited sensitivity of such testing to reveal all variations that may occur and affect the chemical, physical and microbial characteristics of the product. Each step of the manufacturing process must be controlled to the extent necessary to assure that the product meets established specifications. The concept of process validation is a key element in assuring that these quality assurance goals are met.

Validation is the: "Action of proving, in accordance with the principles of good manufacturing practice, that any procedure, process, equipment, material, activity or system actually leads to the expected results". The firm, Albert David, follows all the Components of Good Manufacturing Practice to manufacture the pharmaceutical products in its plant. Process validation is a component of GMP. It is complementary to and not a replacement for other aspects of GMP, such as personnel training records, standard operating procedures, adequate batch documentation, and good quality management system. Before a process can be properly validated, the equipments, facilities, and services used in the process must themselves be validated. Such an operation, often called qualification, is, therefore, an

integral part of process validation which, in turn, is part of GMP. Qualification is usually divided into installation qualification (IQ) and operational qualification (OQ).

The firm has designed its own validation programme and performs the followings for the process validation. The qualifications programme is co-ordinated by means of a written general plan, called a Validation Master Plan. The administration of the qualification programme is normally by means of a Validation Committee, comprised of representations of the different disciplines involved with the programme. These representations must be from departments such a pharmaceutical productions or operations, quality assurance, microbiological quality control, analytical quality control, pharmaceutical development, engineering, and maintenance. The committee approves and issues written protocols, and reviews the data obtained against the results expected, that is, the acceptance criteria, to approve or reject the qualification on validation.

PROCESS VALIDATION DATA

The following datas are collected for the process validation from the manufactured batches of product, controlling the critical parameters and thoroughly testing for compliance to the specification throughout the process. The validity of a manufacturing process can be verified only by laboratory studies of the finished product produced by the same process. Therefore, documentation of the successful completion of such studies is a basic requirement for determining whether a process is suitable for its intended application.

EVALUATION DATA OF PROCESS VALIDATION

Product : MF III of Switzerland HP Injection

Batch No.	Description Colourless	Extractable volume Not less than 2.0 ml	pH 6.0 to 7.5	Heavy metal ▪ 10ppm	Arsenic ▪ 1ppm	Toxicity No death of mice	Sterility No microbial growth	HIV antibody, HBS antigen & HCV antibody Absent	Total Nitrogen Content ▪ 0.08%w/v	Actual yield Number of Ampoule
9P01062	Colourless	2.14 ml	6.92	▪ 10ppm	▪ 1ppm	Zero death of mice	No microbial growth	Absent	0.0153%	103100
9P01063	Colourless	2.15 ml	6.74	▪ 10ppm	▪ 1ppm	Zero death of mice	No microbial growth	Absent	0.0144%	103280
9P01064	Colourless	2.13 ml	6.84	▪ 10ppm	▪ 1ppm	Zero death of mice	No microbial growth	Absent	0.0146%	101100
9P01065	Colourless	2.16 ml	6.82	▪ 10ppm	▪ 1ppm	Zero death of mice	No microbial growth	Absent	0.0140%	102880
9P01066	Colourless	2.15 ml	6.93	▪ 10ppm	▪ 1ppm	Zero death of mice	No microbial growth	Absent	0.0148%	102700
9P01067	Colourless	2.14 ml	6.80	▪ 10ppm	▪ 1ppm	Zero death of mice	No microbial growth	Absent	0.0154%	103080
9P01068	Colourless	2.15 ml	6.90	▪ 10ppm	▪ 1ppm	Zero death of mice	No microbial growth	Absent	0.0152%	101100
9P01069	Colourless	2.14 ml	6.95	▪ 10ppm	▪ 1pp	Zero death of	No microbial	Absent	0.0147%	103280

					m	mice	growth			
9P01070	Colourless	2.16 ml	6.88	▪ 10ppm	▪ 1ppm	Zero death of mice	No microbial growth	Absent	0.0145%	104200
9P01071	Colourless	2.15 ml	6.86	▪ 10ppm	▪ 1ppm	Zero death of mice	No microbial growth	Absent	0.0144%	103360

P4. CONTROL OF EXCIPIENTS

P4.1. Specifications: Annexure XII

P4.2. Analytical Procedures: Annexure XIII

P4.3. Validation Of Analytical Procedures:

The analytical procedures for the control of excipients are followed as per BP monographs. The users of these methods are not required to validate accuracy and reliability of these methods, but merely verify this suitability under actual conditions of use.

P4.4. Justification Of Specifications: Pharmacoepial Requirement

P4.5. Excipient of Human or Animal Origin: No

P4.6. Novel Excipients: No

P5. CONTROL OF FINISHED PRODUCTS

P5.1 Specification : Annexure I

P5.2 Analytical Procedures: Annexure II

P5.3 Validation of Analytical Procedures:

VALIDATION OF ANALYTICAL PROCEDURE FOR MF III OF SWITZERLAND HP INJECTION

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. Typical analytical performance characteristics that should be considered in the validation of the types of methods described in the document are Accuracy, Precision, Specificity, Detection Limit, Quantitation Limit, Linearity and Range. Revalidation of analytical procedures may be necessary in the following circumstances: changes in the synthesis of the drug substances; changes in the composition of the drug product; and changes in the analytical procedure.

For validation of analytical procedures of MF III of Switzerland HP Injection five batches were included in these studies and were identified namely by 9P01062, 9P01063, 9P01064, 9P01065 and 9P01066.

The following Specifications and Methods of Analysis for MF III of Switzerland HP Injection were used for the validation studies.

Validation of in-house methods of analysis for MF III of Switzerland HP injection.

Description : (Limit- Colourless clear liquid)

Results : All five batches of MF III of Switzerland HP Injection met the monograph specification.

Discussion : No problems were encountered in conduction this test.

Identification : (Limit- Chromatographic analysis of amino acid profile should match with the standard HPLC chromatogram)

Results : All five batches of MF III of Switzerland HP Injection met the monograph specification.

Discussion : The test present no analytical problems.

Extractable Volume : (Limit- Not less than 2.0 ml)

Results : MF III of Switzerland HP Injection	Test-1	Test-2	Test-3
Batch 9P01062	2.10 ml	2.12 ml	2.08 ml
Batch 9P01063	2.12 ml	2.12 ml	2.10 ml
Batch 9P01064	2.14 ml	2.12 ml	2.15 ml
Batch 9P01065	2.11 ml	2.10 ml	2.12 ml
Batch 9P01066	2.12 ml	2.11 ml	2.13 ml

Discussion : All five batches of MF III of Switzerland HP Injection met the monograph limit. There were no problems associated with this test.

pH : (Limit- 6.00 to 7.50)

Results : MF III of Switzerland HP Injection	Test-1	Test-2	Test-3
Batch 9P01062	pH-6.80	pH-6.82	pH-6.81
Batch 9P01063	pH-6.90	pH-6.91	pH-6.90
Batch 9P01064	pH-7.02	pH-7.03	pH-7.01
Batch 9P01065	pH-6.92	pH-6.92	pH-6.93
Batch 9P01066	pH-6.87	pH-6.88	pH-6.87

Discussion : All five batches of MF III of Switzerland HP Injection conformed to the monograph limits. The test present no analytical problems.

Specific gravity : (Limit- 1.0020 to 1.0060)

Results : MF III of Switzerland HP Injection	Test-1	Test-2	Test-3
Batch 9P01062	1.0024	1.0025	1.0023
Batch 9P01063	1.0025	1.0025	1.0024
Batch 9P01064	1.0024	1.0025	1.0023
Batch 9P01065	1.0023	1.0024	1.0023
Batch 9P01066	1.0025	1.0025	1.0026

Discussion : All five batches of MF III of Switzerland HP Injection met the monograph limit. No problems were encountered in conducting this test.

Heavy Metals : (Limit-Not more than 10 ppm).

Results : All five batches of MF III of Switzerland HP Injection met the monograph limits.

Discussion : There were no problems associated with this test.

Arsenic : (Limit-Not more than 1 ppm).

Results : All five batches of MF III of Switzerland HP Injection met the monograph limits.

Discussion : No problems were encountered in conducting this test.

Sulphated Ash : (Limit- Not more than 0.1% w/v).

Results : MF III of Switzerland HP Injection	Test-1	Test-2	Test-3
Batch 9P01062	0.031%	0.033%	0.032%
Batch 9P01063	0.034%	0.034%	0.033%
Batch 9P01064	0.032%	0.033%	0.031%
Batch 9P01065	0.035%	0.034%	0.035%
Batch 9P01066	0.032%	0.033%	0.033%

Discussion : There were no problems associated with this test.

Abnormal Toxicity : (Limit- None of mice dies within 24 hours).

Results : All five batches of MF III of Switzerland HP Injection met the monograph limits.

Discussion : No problems were encountered in conducting this test.

Sterility : (Limit-No microbial growth in sterility test media)

Results : MF III of Switzerland HP Injection	Test-1	Test-2	Test-3
Batch 9P01062	No growth	No growth	No growth
Batch 9P01063	No growth	No growth	No growth
Batch 9P01064	No growth	No growth	No growth
Batch 9P01065	No growth	No growth	No growth
Batch 9P01066	No growth	No growth	No growth

Discussion : All five batches of MF III of Switzerland HP Injection complies with the requirements of the monograph. No problems were encountered in conducting this test.

HIV Antibody : (Limit- HIV Antibody must be absent)

Results : MF III of Switzerland HP Injection	Test-1	Test-2	Test-3
Batch 9P01062	Absent	Absent	Absent
Batch 9P01063	Absent	Absent	Absent
Batch 9P01064	Absent	Absent	Absent
Batch 9P01065	Absent	Absent	Absent
Batch 9P01066	Absent	Absent	Absent

Discussion : All five batches of MF III of Switzerland HP Injection met the specified limits. There were no problems associated with this test.

Hepatitis B Surface Antigen : (Limit-Hepatitis B Surface Antigen must be absent).

Results : MF III of Switzerland HP Injection	Test-1	Test-2	Test-3
Batch 9P01062	Absent	Absent	Absent
Batch 9P01063	Absent	Absent	Absent
Batch 9P01064	Absent	Absent	Absent
Batch 9P01065	Absent	Absent	Absent
Batch 9P01066	Absent	Absent	Absent

Discussion : All five batches of MF III of Switzerland HP Injection met the requirements of the monograph. No problems were encountered in conducting this test.

HCV Antibody : (Limit-HCV Antibody must be absent)

Results : MF III of Switzerland HP Injection	Test-1	Test-2	Test-3
Batch 9P01062	Absent	Absent	Absent

Batch 9P01063	Absent	Absent	Absent
Batch 9P01064	Absent	Absent	Absent
Batch 9P01065	Absent	Absent	Absent
Batch 9P01066	Absent	Absent	Absent

Discussion : All five batches of MF III of Switzerland HP Injection met the requirements of the monograph. There were no problems associated with this test.

Assay for Total Nitrogen : (Limit- Total Nitrogen is not more than 0.08%w/v)

Results : MF III of Switzerland HP Injection

	Batch 9P01062	Batch 9P01063	Batch 9P01064	Batch 9P01065	Batch 9P01066
A)	0.0150%	0.0140%	0.0152%	0.0145%	0.0144%
B)	0.0155%	0.0144%	0.0147%	0.0147%	0.0140%
C)	0.0145%	0.0136%	0.0154%	0.0150%	0.0138%
D)	0.0153%	0.0135%	0.0155%	0.0140%	0.0146%
E)	0.0147%	0.0145%	0.0146%	0.0143%	0.0148%
Average	0.0150%	0.0140%	0.01508%	0.0145%	0.01432%
S.D.	0.00032	0.000316	0.000316	0.000316	0.000316
Relative S.D.	2.1333%	2.2571%	2.0968%	2.1806%	2.2081%
Range	0.0145% to 0.0155%	0.0135% to 0.0145%	0.0146% to 0.0155%	0.0140% to 0.0150%	0.0138% to 0.0148%

Discussion : In the assay for total nitrogen the average assay values, standard deviations, relative standard deviations and ranges produced were quite satisfactory. All five batches of MF III of Switzerland HP Injection met the monograph limits. No problems were encountered in conducting this test.

P5.4 Batch Analysis (Minimum 2 batches): Annexure III

P5.5 Characterisation of Impurities: NA

P5.6 Justification of Specification(s):

The specification of the finished product is designed to identify, characterize and ensure presence of specified constituents in the product and to meet the microbiological attributes with respect to sterility in conformity with pharmacoepial requirements.

P6. REFERENCE STANDARDS OR MATERIAL INFORMATIONS:

Not Required

P7. CONTAINER CLOSURE SYSTEM (Referring to Part I section C2):

2 mL glass ampoule

P8. STABILITY DATA (Minimum 2 batches):

Annexure XIV

P9. PRODUCT INTERCHANGEABILITY / EQUIVALENCE EVIDENCE (If any):

NA

ANNEXURE I

Laboratoires Dom-AVMM SUISSE
6340 Baar Schweiz-Suisse-Switzerland

TEL. # : +41 44 22742424
FAX. # : +41 44 22742424

QUALITY CONTROL DEPARTMENT

Document : FINISHED PRODUCT SPECIFICATION	
Product Name: MF III OF SWITZERLAND HP INJECTION	
Generic Name : HUMAN PLACENTA	**Pack : Clear Glass Vial Bottles**
Standards : In-House	**Shelf Life : 6 years**

SERIAL NO.	PARAMETERS	SPECIFICATION
1.	DESCRIPTION	Colourless clear liquid.
2.	IDENTIFICATION	Chromatographic analysis of amino acid profile should be matching with the standard HPLC chromatogram
3.	EXTRACTABLE VOLUME	Not less than 2.0 mL
4.	pH	6.8 to 7.2
5.	SPECIFIC GRAVITY	1.001 to 1.0060
6.	HEAVY METAL	Not More Than 10 ppm
7.	ARSENIC	Not More Than 1 ppm
8.	SULPHATED ASH	Not More Than 0.1 %
9.	ABNORMAL TOXICITY	Should pass the test
10.	STERILITY	Should pass the test
11.	ASSAY Each mL contains	Total Nitrogen : Not More Than 0.08 % (w/v)
12.	HIV Antibody, HBS Antigen & HCV Antibody	Absent

ANNEXURE II

METHOD OF ANALYSIS OF MF III OF SWITZERLAND HP INJECTION

1. Identification :

The amino acid profile chromatogram of the test sample has to be comparable to that of Standard Sample of MF III of Switzerland HP Injection.

Method of amino acid profile development (By HPLC)
HPLC System (model : LC – 10A, make : Shimadzu)

1.1 The system employs derivatisation reaction of amino acids with OPA (ophthalaldehyde).
1.2 The system includes HPLC equipment as a main unit and can analyze free amino acids as well as hydrolyzed amino acids.
Stationary Phase

The system uses styrene-diviny1 benzene copolymer with sulfonic acid, i.e. strongly acidic cation exchange resin, (sodium type) for separation.

Detector

Fluorescence Detector (model : RF – 10AXL)

Reagents

i) Sodium Citrate
ii) n-Caprylic Acid
iii) Ethanol 99.5%
iv) Perchloric Acid 60%
v) Sodium Carbonate
vi) Potasium Sulphate
vii) o-phthalaldehyde
viii) Brij-35
ix) Sodium Hypochlorite
x) 2-Mercaptoethanol
xi) Sodium Hydroxide
xii) Distilled water HPLC grade

Mobile Phases

Chemical Composition :

	Liquid A	Liquid B	Liquid C
PH	3.20	10.00	pH not adjusted
Sodium Citrate $2H_2O$ (g)	58.8	58.8	-
Sodium Hydroxide (g)	-	-	4
Boric Acid (g)	-	12.4	-
Ethanol (99.5%) (ml)	210	-	-
Perchloric Acid (ml)	50	-	-
4N NaOH solution (ml)	-	30 ml	-
Final volume (In liter)	3	1	0.5

Procedure for preparing 4N sodium Hydorxide aqueous solution for pH adjustments.

Weigh out 32g of NaOH and dissolve in 200ml of distilled water.

Conduct pH fine adjustment for the solutions A and B accurately using digital pH meter and standard buffer solutions.

Filtration Of Mobile Phase

Separately filter mobile phases A,B and C through 0.45 micron membrane filter. Take care so that the solutions do not mix with each other and that they are not contaminated with ammonia.

Preparation Of Derivatization Reaction Reagent :

Buffer

Chemical Composition	Quantity In Gms	Molar Concentration
Sodium Carbonate	122.2	0.384M
Boric Acid	40.7	0.216M
Potassium Sulphate	56.4	0.108M

Dissolve the above reagents in water to make 3 liter solution. Use this buffer without pH fine adjustment.

Brij-35, 10% solution (polyoxyethelene lauryl ether)

Weigh out 10g of Brij-35 to make 100 ml of solution. Put the bottle in warm water to facilitate dissolution of Brij-35.

Reaction Reagent A [Sodium hypochlorite solution (NaClO solution)]

Take 500 ml of boric acid-carbonic acid buffer by a measuring cylinder. And 0.2ml of commercially available sodium hypochlorite aqueous (effective chlorine concentration around 7-10%) solution. Mix them thoroughly and filter through 0.45 micron membrane filter.

Reaction Reagent B (0.08% OPA solution)

Chemical Composition

Reagent	Quantity
OPA	400 mg
Ethanol	7 ml
2-mercaptoethanol	1 ml
10% Brij – 35 solution	2 ml
	Add buffer to make 500 ml of solution

Preparation Of Diluent For Sample

Weight out 9.8 g of sodium citrate. Ad[sd/plx/04]d about 400ml of distilled water to dissolve the sodium citrate. Then, add 8ml of perchloric acid and 0.05 ml of n-caprylic acid. Add distilled water to make 500ml solution. Then, adjust the pH of the solution to 2.20 by adding perchloric acid.

Sample Solution

Use MF III of Switzerland HP Injection as it is.

Chromatographic System

A. Column

Separation column : Shimpack ISC-07 / S1504 Na (4mm ID × 15 cm long)
(Make : Shimadzu)

Guard column : Shimpack ISC-07 N a (4 mm ID × 15 cm long)
(Make : Shimadzu)

Ammonia trap column: Shimpack ISC-30 /S0504 Na(4 mm ID × 15 cm long)
(Make : Shimadzu)

B. Run Parameters

Total flow of mobile phase	: 0.300 mL/ minute
Pressure Maximum	: 100.0 kgf / cm^2
Pressure Minimum	: 0.0 kgf / cm^2
Run Time	: 90 minutes
Reaction Reagent Flow Rate	: 0.2 mL / minute (each)
Column Oven Temperature	: 55 °C

C. Detector Settings

Excitation wavelength : 348 nm

Emission wavelength : 450 nm

s

Spectrum type : Emission

Gain : x 4

Sensitivity : Low

Scan Speed : Fast

Sampling Period : 500 mili second

Delay : 0 minute

D. Gradient program

Time	Event	Value
0.01	B..Conc	0.0
7.00	T.Flow	0.3
12.00	T.Flow	0.1
17.00	B..Conc	0.0
24.00	T.Flow	0.1
24.01	T.Flow	0.2
29.00	T.Flow	0.3
35.00	B..Conc	16.0
40.00	B..Conc	16.0
40.01	B..Conc	60.0
45.01	T.Flow	0.3
50.00	B..Conc	100.0
52.00	T.Flow	0./3

52.02	T.Flow	0.1
55.00	T.Flow	0.1
56.00	T.Flow	0.3
58.00	T.Flow	0.3
60.00	B..Conc	100.0
60.01	B..Conc	0.0
60.02	SV (Pump A)	1.0
65.00	SV (Pump A)	0.0
90.00	STOP	0.0

2. EXTRACTABLE VOLUME

Withdraw completely the solution of 5 containers using 5 mL syringe and measure the volume using a 10 mL measuring cylinder. Determine the average volume.

3 pH

Take about 50 mL of sample in a 100 mL beaker. Immerse the electrode of the pH meter into the solution and measure the pH of the sample at 25°C ± 2°C

4. Specific Gravity

Select a thoroughly clean and dry pycnometer and weigh it. Adjust the temperature of the water to about 20 ^{O}C and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25 ^{O}C, remove any excess of the water and weigh.Again dry the pycnometer. Adjust the temperature of the sample to about 20 ^{O}C and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25 ^{O}C, remove any excess of the sample and weigh. Calculate the specific gravity using the following formula :

$$\text{Specific Gravity} : \frac{\text{Weight of (pycnometer + sample) -- Weight of pycnometer}}{\text{Weight of (pycnometer + water) -- Weight of pycnometer}}$$

5. Heavy Metals

Prepare test solution and standard solution as directed below :

Standard solution :

Into a 50-mL Nessler cylinder pipette 1.0 mL of lead standard solution (20 ppm Pb) and dilute with water to 25 mL. Adjust with dilute acetic acid or dilute ammonia solution to a pH between 3.0 and 4.0, dilute with water to about 35 mL and mix.

Test solution:

Place 2.0 g of the sample into a 50-mL Nessler cylinder and make the volume to 25 mL with water. Adjust with dilute acetic acid or dilute ammonia solution to a pH between 3.0 and 4.0, dilute with water to about 35 mL and mix.

Procedure:

To each of the cylinders containing the standard solution and test solution respectively add 10 mL of freshly prepared hydrogen sulphide solution, mix, dilute to 50 mL with water, allow to stand for 5 minutes and view downwards over a white surface; the colour produced with the test solution is not more intense than that produced with the standard solution.

6. Arsenic

Into the bottle or conical flask introduce 10.0 g of the substance being examined; add 50 mL of water and 10 mL of stannated hydrochloric acid. Into the resulting solution add 5 mL of 1M potassium iodide and 10 g of zinc AsT.

Immediately assemble the arsenic apparatus and immerse the flask in a water bath at a temperature such that a uniform evolution of gas is maintained. After 45 minutes any stain produced on the mercuric chloride paper is not more intense than that obtained by treating in the same manner 1.0 mL of arsenic standard solution (10ppm As) dilute to 50 mL with water.

7. Sulphated Ash

Heat a silica crucible at 800°C for 10 minutes, allow to cool in a desiccator and weigh. Transfer to the crucible 1 g of the substance being examined and weigh the crucible and the contents accurately. Ignite, gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 mL of sulphuric acid, heat gently until the white fumes are no longer evolved and ignite in a muffle furnace, at a temperature $800^{O} \pm 25^{O}$ C, until all black particles have disappeared and continue heating until a white or at most greyish residue is produced. Allow to cool the crucible in a desiccator to room temperature and weigh again. Repeat the operation until two

successive weighings do not differ by more than 0.5 mg. Calculate the percentage of sulphated ash using the following formula :

$$\text{Sulphated Ash} : \frac{[\text{Final weigh of crucible} - \text{Initial weigh of crucible}]}{\text{Weight of sample taken in g}} \times 100\ \%$$

8. Abnormal Toxicity

Dilute 0.2 mL of the sample to 10 mL with water for injection. Inject travenously 0.2 mL of this Solution per 20 gm body weight to each of 5 mice that have been deprived from the food for not less than 17 hours After the injection allow the mice access of food and water.

The sample passes the test if none of the mice dies within 24 hours. If one of the mice dies within 24 hours, repeat the test. The sample passes the test if none of the second group of mice dies within 24 hours.

9. STERILITY

Materials And Equipments Required

1. Laminar flow work station.
2. Manifold Filtration unit made of stainless steel.
3. Vacuum pump for filtration.
4. Membrane filter :
5. Manufacturer : Pall Life Sciences / Millipore / Sartorius.
   Catalog No. : As per individual manufacturer.
   Diameter : 47 mm
   Pore Size : 0.45 µm
6. Autoclave for sterilization.
7. Ampoule cutter, membrane filtration holder, scissor, forceps, gloves, mask and silicone adapter. All these items should be sterilized before sterility testing.

8. Peptone, Bacteriological grade, Himedia (RM001)
9. Fluid Thioglycollate Medium, Himedia (M009)
10. Soyabean Casein Digest Medium, Himedia (MO11)
11. Isopropyl Alcohol (70%).
12. Water for Injection I.P.
13. Culture tube, Capacity : 200 mL; Borosil; Without Rim
14. Cellophane paper and Brown paper for wrapping the items before sterilization.
15. Isopropyl alcohol 70% dispenser.
16. Membrane Filter Holder Unit :

This is consisting a closed reservoir with supporting porous metallic screen. Place the membrane properly on the supporting screen and assemble the entire unit and wrap with cellophane paper and Brown paper. Sterilize the assembled unit at 121^0C at 15 lbs for15 minutes.

17. BOD Incubator having the temperature range 20°C - 25° C.
18. Incubator having the temperature range 30°C - 35°C.

Preparation of Culture Media

1.Soyabean Casein Digest Medium, Himedia (M011)

Dissolve 30 g of Soyabean Casein Digest Medium in 1000 mL of distilled water by boiling. Fill 100 mL of the prepared solution in 200 mL culture tubes and plug it properly with non-absorbent cotton. Sterilize in an autoclave at 121°C at 15 lbs for 15 minutes. Cool the tube and incubate for 7 days before actual work.

2. Sterile diluting fluid

Dissolve 10 g of Peptone, Bacteriological (Himedia, RM001) in 1 liter of water for Injection .

Adjust the pH to 7.1 ± 0.2. Distribute10 mL of the diluting fluid into each of the test tubes and sterilize at 121°C at 15 lbs for 15 minutes. Cool the flasks and incubate for 48 hours before actual work.

3. Fluid Thioglycollate Media, Himedia (M009)

Dissolve 29.75 g of Fluid Thioglycollate Media in 1000 mL of distilled water by boiling. Fill 100 mL of the prepared solution in 200 mL culture tubes and plug it properly with non-absorbent cotton. Sterilize in an autoclave at 121°C at 15 lbs for 15 minutes. Cool the tubes and incubate for 7 days before actual work.

Method of analysis

Clean and disinfect the exterior and interior surfaces of Laminar Air-Flow work station, rubber tubing, burner with isopropyl alcohol 70% . Burner should be lighted only after removal of the alcohol. Under aseptic condition on a laminar flow bench, make access to the contents in a suitable manner and transfer aseptically minimum 1.5 mL of sample from 20 ntainers to the membrane filter assembly and filter immediately with aid of vacuum pump. Rinse the membrane filter with 10 mL of sterile diluting

fluid. Aseptically remove the membrane and cut into two equal parts. Immerse one part in one 100 mL of fluid thioglycollate medium and incubate at 30°C - 35°C for the detection of aerobic and anaerobic bacteria. Similarly, immerse the other part of the membrane in one 100 mL of soyabean casein digest medium and incubate at 20°C - 25°C for the detection of fungi and aerobic bacteria. All the tubes are incubated for 14 days.

Procedure of Negative Control

Under aseptic condition, add 10 mL of sterile diluting fluid without the test substance to one of the membrane filter units (with 0.45 micron membrane filter) which is also sterilized along with other units and filter with the aid of vacuum. Treat the membrane as prescribed in the test procedure.

Procedure of Positive Control

Filter a known contaminated solution containing very few microorganisms of varying types (eg. *Staphylococcus aureous, Bacillus subtilis, Candida albicans*) individually through a previously sterilized 0.45 micron porosity membrane filtration unit. Treat the membrane as prescribed in the test procedure . The positive result (proper growth) for the test membrane and the negative result (no growth) for the filtrate confirm the adequacy of the technique being used.

Observation and interpretation of results

At intervals during the incubation period and at its conclusion examine the media for macroscopic evidence of microbial growth. If no evidence of microbial growth is found, the product to be examined complies with the test for sterility.
If evidence of microbial growth is found the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined. The test may be considered invalid only when one or more of the following conditions are fulfilled:

a) the data of the microbiological monitoring of the sterility testing facility shows a fault;
b) a review of the testing procedure used during the test in question reveals a fault;

c) microbial growth is found in the negative controls;

d) after determination of the identity of the micro-organisms isolated from the test, the growth of this species or these species may be ascribed unequivocally to faults with respect to the material and/or the technique used in conducting the sterility test procedure.

If the test is declared to be invalid it is repeated with the same number of units as in the original test.

If no evidence of microbial growth is found in the repeat test the product examined complies with the test for sterility. If microbial growth is found in the repeat test the product examined does not comply with the test for sterility.

10. ASSAY

Equipment Required

1. Kjeldalh flask 250 mL : 1 No.
2. Distillation set-up : 1 Set.
3. Glass funnel : 1 No.
3. Conical flask 250 mL : 1 No.
4. Standard burette 50 mL : 1 No.
5. Glass bead : 2 Nos.

Reagents Required

1. Potassium sulphate / sodium sulphate anhydrous, A.R.
2. Cupric sulphate, AR
3. Purified water
4. Sulphuric acid solution, 0.01N
5. Sodium Hydroxide solution 50% w/v
6. Boric Acid solution, 4% w/v
7. Methyl red - Mathylene blue indicator solution

Mix 10mL of methyl red solution(0.1% in alcohol) with 10mL of methylene blue solution (0.125%in alcohol).

Procedure

Transfer accurately 10 mL of MF III of Switzerland HP injection into a 250 mL kjeldahl flask . Add 2 gm of powdered potassium sulphate 200 mg of powdered cupric sulphate and 10 mL of sulphuric acid . Add two glass beads through the wall of kjeldahl flask at 45° in a fuming chamber. Place a funnel on the month of kjeldahl flask. Heat the mixture gently (at low flame), keeping the temperature below the boiling point until frothing has ceased. Increase the heat, until the acid boils briskly and continue the heating until the solution has become clear green in colour or almost colourless for 30 minutes.

Allow to cool, add 100 mL of purified water . Mix the contents of the flask and cool it again by keeping the flask in crushed ice. Make the distillation set ready by assembling different parts of distillation assembly. Add cautiously 50 mL of 50 % sodium hydroxide solution slowly in such a manner that the solution flows down the inner side of the kjeldahl flask to form a layer under the acid solution. Without delay, connect the

flask to a kjeldahl connecting bulb(trap), previously attached to a condenser. The delivery tube from this condenser should be dipped into a mixture of 25 mL of 4 % boric acid solution and 75 mL of distilled water kept in a conical flask .
Heat the flask gradually for distillation until about four-fifth of the content of the flask has distilled over. Rinse the condenser with purified water and transfer all the rinsing solution to the conical flask. Add 3 to 4 drops of methyl red-methylene blue solution to the boric acid solution. Titrate with 0.01N sulphuric acid.

Each mL of 0.01N sulphuric acid is equivalent to 0.0001401 g of nitrogen.

$$\text{Calculation : Content of Total Nitrogen} = \frac{0.0001401 \times F \times 100 \times \text{Titre value}}{10} \text{ \% (w/v)}$$

11. HIV ANTIBODY, HCV ANTIBODY AND HBS ANTIGEN

List of Accessories

1. ELISA Kit for detection of
   HIV-1 and HIV-2 Antibodies
   HCV Antibodies
   HBs Antigen
2. Distilled water
3. Micropipette of 100 µl and 1000 µl capacity and related disposable tips.
4. Microplate washing instrument
5. Measuring cylinder of 100 ml.
6. Disposable absorbent paper
7. Disposable gloves
8. Autoclavable 5 lit. beaker
9. Microplate ELISA reader

HBs antigen testing method

Bring all the reagents to room temperature before use except the color reagent. Store the color reagent at 2-8^{0}C till use. Then remove required number of microwells from the packet and label them appropriately. In each run use one reaction blank, three negative controls and one positive control.

Fill all the wells with 100 µl of sample diluent and add 100 µl of negative control, positive control and test solutions to the respective wells. Keep the first well as the reaction blank. Cover all the strips with adhesive strip cover after shaking. Incubate the strips for 1 hour at room temperature (25^{0}C – 40^{0}C).

After completion of the incubation time discard the adhesive strip cover and aspirate the contents of the wells. Then add 50 µl of conjugate stabilizer first, followed by 100

µl of conjugate to each well except blank. Then agitate all strips gently for 5- 10 seconds to mix the contents of the micro wells. After that incubate the strips at room temperature for 30 minutes.

After completion of the incubation decant the content of the wells into a waste container by the autowasher. Then fill all wells with diluted washing buffer (approx 350 µl) and soak for 30 seconds and again wash. Repeat the washing four times more. Finally remove excess fluid by tapping on a disposable absorbent paper.
Then add 100 µl of color reagent in each well and incubate for 30 minutes in dark at room temperature.
After that add 100 µl of stopping solutions in all wells to stop the reaction.

Finally take the absorbance reading bichromatically at 450 nm using 630 nm as reference wave length with the help of ELISA reader.

Calculation for determination of Cut off value

Cut off value is the mean OD of negative control (NCX) + 0.100

Interpretation of result

All the samples with the absorbance less than that of Cut off value should be considered negative and the absorbance is equal to or greater than that of Cut off value should be considered positive for HBs.

Note:

(i) The absorbance of negative control must be less than 0.100
(ii) The absorbance value of positive control must be greater than 1.500

(iii) The assay run is considered to be valid if the absorbance of the negative control, positive control are within the acceptable range. If they are not, technique or reagents should be suspected to be at fault and the assay should be repeated.

HIV 1&2 Antibody testing method

Bring all reagents and samples to room temperature except the colour reagent which is to be stored at 2-8^{0}C till use. Then remove required numbers of micro wells from the packet and label them appropriately. Keep the blank well empty. Use reaction blank, three negative and one positive control for each run.

Fill 200 µl of sample diluent in all of the wells except the reaction blank well. Then add 10 µl of negative, positive and test solution to the respective wells. Cover all the strips with adhesive strip cover after gentle shaking and incubate it for 30 minutes at room temperature (20^{0}C to 30^{0}C).

Discard the adhesive strip cover after the incubation period and decant all the contents of the wells into a waste container by auto washer. Fill the diluted washing buffer (approx 350 µl) and allow it to soak for 30 seconds. Then decant it in the waste container. Repeat the same for four more times and finally remove the excess fluid by tapping the strips on a disposable absorbent paper.

Add 50 µl of conjugate to each of the wells except the reaction blank well immediately after tapping. Cover the strips after gentle mixing of the contents of the microwells and incubate for 30 minutes at room temperature (20^{0}C -30^{0}C).

Discard the adhesive strip cover after the incubation period and wash five times by the diluted washing buffer as discussed above. Add 100 µl of colour reagent into each well including the reaction blank and keep 15 minutes in dark.

After that, add 100 µl of stopping solution in all the wells including the reaction blank control to stop the reaction.
Finally take the absorbance reading bichromatically at 450 nm by using 630 nm as reference wave length by the ELISA reader.

Calculation for determination of Cut off value

Cut off value is the mean OD of negative control (NCX) + 0.225

Interpretation of result

All the samples with the absorbance less than that of Cut off value should be considered negative for anti HIV and the absorbance is equal to or greater than that of Cut off value should be considered positive for anti HIV.

Note:

(i) The absorbence of individual negative control must be greater than - 0.010 O.D units &less than or equal to 0.200 O.D units. One negative control may be discarded if it is outside of this range. Then mean negative control (NCX) is calculated from the two remaining values instead of three negative control values.
(ii) The absorbance value of positive control should be atleast 1.000.
(iii) The absorbance value (O.D) of reagent blank should be between 0.000 to 0.100
(iv) The assay run will be valid if the O.D values of positive control, negative control and reagent blank are within the acceptable range. If they are not, technique or reagents should be suspected to be at fault and the assay should be repeated.

HCV antibody testing method

Bring all the reagents in room temperature before use except colour reagent (to be stored at 2-8^0 C till use). Remove the required number of antigen coated microwells/ strips from the packet. Label the wells appropriately. Leave the reaction blank well empty. Use reaction blank, three negative and one positive control for each run.

Fill 200 µl of sample diluent in all of the wells except the reaction blank well. Then add 10 µl of negative, positive and test solution to the respective wells. Cover all the strips with adhesive strip cover after gentle shaking and incubate it for 30 minutes at room temperature (20^{0}C to 30^{0}C).
Discard the adhesive strip cover after the incubation period and decant all the contents of the wells into a waste container by auto washer. Fill the diluted washing buffer (approx 350 µl) and allow it to soak for 30 seconds. Then decant it in the waste container. Repeat the same for four more times and finally remove the excess fluid by tapping the strips on a disposable absorbent paper.

Add 50 µl of conjugate to each of the wells except the reaction blank well immediately after tapping. Cover the strips after gentle mixing of the contents of the microwells and incubate for 30 minutes at room temperature (20^{0}C -30^{0}C).

Discard the adhesive strip cover after the incubation period and wash five times by the diluted washing buffer as discussed above. Add 100 µl of colour reagent into each well including the reaction blank and keep 15 minutes in dark.

After that, add 100 µl of stopping solution in all the wells including the reaction blank control to stop the reaction.

Finally take the absorbance reading bichromatically at 450 nm by using 630 nm as reference wave length by the ELISA reader.

Calculation for determination of Cut off value

Cut off value is the mean OD of negative control (NCX) + 0.225

Interpretation of result

All the samples with the absorbance less than that of Cut off value should be considered non-reactive for anti HCV and the absorbance more than that of Cut off value should be considered reactive for anti HCV.

Note:
(i) The absorbance of individual negative control must be greater than 0.000 O.D units &less than or equal to 0.200 O.D units. One negative control may be discarded if it is outside of this range. Then mean negative control (NCX) is calculated from the two remaining values instead of three negative control values.

(ii) The absorbance value of positive control should be atleast 1.0.

(iii) The absorbance value (O.D) of reagent blank should be between 0.000 to 0.100

(iv) The assay run will be valid if the O.D values of positive control, negative control and reagent blank are within the acceptable range. If they are not, technique or reagents should be suspected to be at fault and the assay should be repeated.

ANNEXURE III

CERTIFICATE OF ANALYSIS

Product Name	MF III OF SWITZERLAND HP INJECTION		
Generic Name	HUMAN MF III OF SWITZERLAND HP	COA Number	AR/4P01001
Batch Number	4P01001	Analysis Date	21.01.2006
Batch Size	240 Liters	Sampled Quantity	200 Ampoules
Manufacturing Date	December 2004	Sampling Date	07.01.2006

Expiry date	December 2010	Reference Number	F/003/04

Test	Specification	Result
Description	Colourless to pale yellow coloured clear liquid.	Pale yellow coloured clear liquid
Identification	Chromatographic analysis of amino acid profile should be matching with the standard HPLC chromatogram	Complies
Extractable Volume	Not less than 2.0 mL	2.14 mL
pH	6.0 to 7.5	6.9
Specific Gravity	1.0010 to 1.0060	1.0022
Heavy Metal	Not more than 10 ppm	Less than 10 ppm
Arsenic	Not more than 1 ppm	Less than 1 ppm
Sulphated Ash	Not more than 0.1 %	0.03 %
Abnormal Toxicity	Should pass the test	Pass
Sterility	Should pass the test	Pass
Assay Total Nitrogen Content :	Not More Than 0.08 %(w/v)	0.011 % (w/v)
HIV Antibody, HBS Antigen & HCV Antibody	Should be absent	Absent

CERTIFICATE OF ANALYSIS

Product Name	MF III OF SWITZERLAND HP INJECTION		
Generic Name	HUMAN MF III OF SWITZERLAND HP	COA Number	AR/4P01002
Batch Number	4P01002	Analysis Date	23.01.2004
Batch Size	240 Liters	Sampled Quantity	100 Ampoules
Manufacturing Date	January 2004	Sampling Date	09.01.2004
Expiry date	December 2005	Reference Number	F/004/04

Test	Specification	Result

Description	Colourless clear solution.	Complies
Identification	Chromatographic analysis of amino acid profile should be matching with the standard HPLC chromatogram	Complies
Extractable Volume	Not less than 2.0 mL	2.12 mL
pH	6.8 to 7.2	6.87
Specific Gravity	1.0010 to 1.0060	1.0025
Heavy Metal	Not more than 10 ppm	Less than 10 ppm
Arsenic	Less than 1 ppm	
Sulphated Ash	Not more than 0.1 %	0.05 %
Abnormal Toxicity	Should pass the test	Pass
Sterility	Should pass the test	Pass
Assay Total Nitrogen Content :	Not More Than 0.08 %(w/v)	0.014 % (w/v)
HIV Antibody, HBS Antigen & HCV Antibody	Should be absent	Absent

ANNEXURE IV

PLACENTA EXTRACT PREPARATION FLOW – CHART

Individually packed Placenta in polythene bag, collected from various from Hospitals, are received, identified and stored in walk-in-cooler maintained at a temperature 2 – 4^0C.

↓

Blood sample of each Placenta is collected by Q. C. for **Elisa Testing**.

Placenta not conforming to the requirements is rejected.

↓

Placenta remains in the cooler for 4 –5 days and taken out for processing after Q.C. release on FIFO basis

↓

Before taking the Placenta for processing, it is physically examined by trained personnel and any Placenta not conforming to requirements of -

- Odour
- Appearance
- Rupture etc.

are discarded.

↓

Placenta drawn from the cooler, after above examination, is kept in WFI for 20 minutes and then rinsed thoroughly with Water for injection

↓

Dressing of Placenta

Water is drained and skilled personnel perform dissection to remove cord veins, blood vessels and fat layers.

↓

Suspending it in required amount of WFI for further processing for cold process extraction

↓

Once the extraction process is over the crude extract is subjected to ultra filtration

↓

Filter using appropriate ultra filtration Media

↓

Autoclave the ultra filtered Extract

↓

Storage overnight

Filtered again through ultra Filtering Media (Lot A, B & C separately)

← Sampling for ELISA testing for (pooled Placenta) lot

Aseptic Membrane Filtration and pooling of all lots into one batch Bulk Extract

← For ELISA testing on a Bulk Batch

Bulk Solution taken for Product Manufacturing

ANNEXURE V

PLACENTA COLLECTION AND STORAGE AT HOSPITALS

Procedure:

1. Placenta from eligible donors are collected at Government approved Hospitals only. The list of Government approved hospitals is attached as Annexure –1.

2. The eligible donors are pregnant mothers registered with any of the approved European Union. Placenta from full-time pregnancy donors,

preferably 1st, 2nd, & 3rd gravida, is collected by fully trained nursing staff/ labor room I/C of the hospital.

3. **Donors suffering from communicable diseases, Hepatitis, HIV, Breech delivery, Twins birth, under weight birth, Burns are not considered.**

4. Placenta from eligible donors are collected immediately after birth & is kept individually in yellow plastic bags, provided by the Company. The bags are labeled with the name of the donor, date & time pregnancy, name of the Hospital and signature of the hospital staff.

5. The bag with the placenta is then transferred to the Refrigerator provided for the purpose to the Hospital by the Company.

6. The placenta collected in a day is removed from the Refrigerator by a Company authorized person and kept in a cool ice box to be transported to the Factory Premises.

7. The delivery of the placenta at Factory is recorded by the I/C Parenteral Department and stored in Walk-in-Cooler maintained at 2-4 degree Centigrade.

ELISA TESTING SPECIFICATION AND PROCEDURE FOR PLACENTA

1. Specification :

HIV-1, 2 Antibody	:	Negative
Hepatitis B Surface Antigen	:	Negative
Hepatitis C Antibody	:	Negative

2. Testing Procedures :

2.1 Stages of In-process Testing :

Individual Placenta blood
Thirty Placenta pooled Extract
Every batch of finished Product (Bulk stage)
-MF III of Switzerland HP Injectables

2.2. Procedure of sampling and storage of the samples:

The above samples are supplied by the respective departments and store all at 2-8^{0}C.

2.3 Accessories:

2.3.1 ELISA Kit for
HIV-1 and HIV-2 Antibodies
HCV Antibodies
HBs Antigen

2.3.2 Distilled water

2.3.3. Micropipette of 100 µl and 1000 µl capacity and related disposable tips.

2.3.4. Plate washing instrument

2.3.5. Measuring cylinder of 100 ml.

2.3.6. Disposable absorbent paper

2.3.7. Disposable gloves

2.3.8. Autoclavable 5 lit. Beaker

2.3.9. Microplate ELISA reader

2.4 Procedure of the HBs antigen testing:

2.4.1 Bring all the reagents to room temperature before use except the color reagent. Store the color reagent at 2-8^{0}C till use. Then remove required number of microwells from the packet and label them appropriately. In each run use one reaction blank, three negative controls and one positive control.

2.4.2 Fill all the wells with 100 µl of sample diluent and add 100 µl of negative control, positive control and test solutions to the respective wells. Keep the first

well as the reaction blank. Cover all the strips with adhesive strip cover after shaking. Incubate the strips for 1 hour at room temperature (25^0C– 40^0C).

2.4.3 After completion of the incubation time discard the adhesive strip cover and aspirate the contents of the wells. Then add 50 µl of conjugate stabilizer first, followed by 100µl of conjugate to each well except blank. Then agitate all strips gently for 5- 10 seconds to mix the contents of the micro wells. After that incubate the strips at room temperature for 30 minutes.

2.4.4 After completion of the incubation decant the content of the wells into a waste container by the autowasher. Then fill all wells with diluted washing buffer (approx 350 µl) and soak for 30 seconds and again wash. Repeat the washing four times more. Finally remove excess fluid by tapping on a disposable absorbent paper.

2.4.5 Then add 100 µl of color reagent in each well and incubate for 30 minutes in dark at room temperature.

2.4.6 After that add 100 µl of stopping solutions in all wells to stop the reaction.

2.4.7 Finally take the absorbance reading bichromatically at 450 nm using 630 nm as reference wave length with the help of ELISA reader.

2.4.8 Calculation for determination of Cut off value:
Cut off value is the mean OD of negative control (NCX) + 0.100

2.4.9 Interpretation of result:
All the samples with the absorbance less than that of Cut off value should be considered negative and the absorbance is equal to or greater than that of Cut off value should be considered positive for HBs.

Note:
(i) The absorbance of negative control must be less than 0.100
(ii) The absorbance value of positive control must be greater than 1.500

The assay run is to be valid if the absorbance of the negative control, positive control are within the acceptable range. If they are not, technique or reagents should be suspected to be at fault and the assay should be repeated.

2.5 <u>Procedure of HIV 1&2 Antibody testing:</u>

2.5.1.Bring all reagents and samples to room temperature except the colour reagent which is to be stored at 2-8^0C till use. Then remove required numbers of micro

wells from the packet and label them appropriately. Keep the blank well empty. Use reaction blank, three negative and one positive control for each run.

2.5.2. Fill 200 µl of sample diluent in all of the wells except the reaction blank well. Then add 10 µl of negative, positive and test solution to the respective wells. Cover all the strips with adhesive strip cover after gentle shaking and incubate it for 30 minutes at room temperature (20^0C to 30^0C).

2.5.3. Discard the adhesive strip cover after the incubation period and decant all the contents of the wells into a waste container by auto washer. Fill the diluted washing buffer (approx 350 µl) and allow it to soak for 30 seconds. Then decant it in the waste container. Repeat the same for four more times and finally remove the excess fluid by tapping the strips on a disposable absorbent paper.

2.5.4. Add 50 µl of conjugate to each of the wells except the reaction blank well immediately after tapping. Cover the strips after gentle mixing of the contents of the microwells and incubate for 30 minutes at room temperature (20^0C -30^0C).

2.5.5. Discard the adhesive strip cover after the incubation period and wash five times by the diluted washing buffer as discussed above. Add 100 µl of colour reagent into each well including the reaction blank and keep 15 minutes in dark.

2.5.6. After that, add 100 µl of stopping solution in all the wells including the reaction blank control to stop the reaction.

2.5.7. Finally take the absorbance reading bichromatically at 450 nm by using 630 nm as reference wave length by the ELISA reader.

2.5.8. Calculation for determination of Cut off value:

Cut off value is the mean OD of negative control (NCX) + 0.225

2.5.9. Interpretation of result:

All the samples with the absorbance less than that of Cut off value should be considered negative for anti HIV and the absorbance is equal to or greater than that of Cut off value should be considered positive for anti HIV.

Note:

(i) The absorbance of individual negative control must be greater than - 0.010 O.D units &less than or equal to 0.200 O.D units. One negative control may be discarded if it is outside of this range. Then mean negative control (NCX) is calculated from the two remaining values instead of three negative control values.

(ii)The absorbance value of positive control should be atleast 1.000

(iii) The absorbance value (O.D) of reagent blank should be between 0.000 to 0.100.The assay run will be valid if the O.D values of positive control, negative control and reagent blank are within the acceptable range. If they are not, technique or reagents should be suspected to be at fault and the assay should be repeated.

2.6 Procedure of the HCV antibody testing:

2.6.1. Bring all the reagents in room temperature before use except colour reagent (to be stored at 2-8^{0}C till use). Remove the required number of antigen coated microwells / strips from the packet. Label the wells appropriately. Leave the reaction blank well empty. Use reaction blank, three negative and one positive control for each run.

2.6.2. Fill 200 µl of sample diluent in all of the wells except the reaction blank well. Then add 10 µl of negative, positive and test solution to the respective wells. Cover all the strips with adhesive strip cover after gentle shaking and incubate it for 30 minutes at room temperature (20^{0}C to 30^{0}C).

2.6.3. Discard the adhesive strip cover after the incubation period and decant all the contents of the wells into a waste container by auto washer. Fill the diluted washing buffer (approx 350 µl) and allow it to soak for 30 seconds. Then decant it in the waste container. Repeat the same for four more times and finally remove the excess fluid by tapping the strips on a disposable absorbent paper.

2.6.4. Add 50 µl of conjugate to each of the wells except the reaction blank well immediately after tapping. Cover the strips after gentle mixing of the contents of the microwells and incubate for 30 minutes at room temperature (20^{0}C -30^{0}C).

2.6.5. Discard the adhesive strip cover after the incubation period and wash five times by the diluted washing buffer as discussed above. Add 100 µl of colour reagent into each well including the reaction blank and keep 15 minutes in dark.

2.6.6. After that, add 100 µl of stopping solution in all the wells including the reaction blank control to stop the reaction.

2.6.7. Finally take the absorbance reading bichromatically at 450 nm by using 630 nm as reference wave length by the ELISA reader.

2.6.8. Calculation for determination of Cut off value:
Cut off value is the mean OD of negative control (NCX) + 0.225

2.6.9. Interpretation of result:

All the samples with the absorbance less than that of Cut off value should be considered non-reactive for anti HCV and the absorbance more than that of Cut off value should be considered reactive for anti HCV.

Note:
(i) The absorbance of individual negative control must be greater than 0.000 O.D units &less than or equal to 0.200 O.D units. One negative control may be discarded if it is outside of this range. Then mean negative control (NCX) is calculated from the two remaining values instead of three negative control values.
(ii) The absorbance value of positive control should be at least 1.0.

(iii) The absorbance value (O.D) of reagent blank should be between 0.000 to 0.100. The assay run will be valid if the O.D values of positive control, negative control and reagent blank are within the acceptable range. If they are not, technique or reagents should be suspected to be at fault and the assay should be repeated.

Abbreviation :, ELISA : Enzyme Linked Immunosorbent Assay
HIV : Human Immuno Deficiency Virus
HCV : Hepatitis C Virus
HBS : Hepatitis B Virus Surface Antigen
O.D : Optical Density.

ANNEXURE VI

SAMPLING PROCEDURE FOR ELISA TESTING AT DIFFERENT STAGES OF IN-PROCESS TESTING OF PLACENTA

Precautions

Use clean, sterile glass tubes for the sampling of the Blood extracted from raw placenta, Pooled extract and in process samples at bulk stage for ELISA Testing. Use gloves while sampling.

Sampling Procedure

1. **Blood samples from Individual Placenta:**

(i)Wash your hands with Isopropyl alcohol between two successive sampling. Clean outer portion of the plastic bag containing placenta using 70% IPA. Puncture the bottom of the bag large enough to get the blood sample out of it. Collect about 2 ml blood sample in individual clean, sterile test tube (squeeze if required) and plug it.
(ii) As soon as the sampling is over seal the cavity of the bag.
(iii) Label the tube with the identification details of the corresponding placenta and store the sample in an ice-box (Temp. 2-8^0 C) and transfer the samples to laboratory.

2. **Bulk stage (30 placenta Pool Sample)**

(i)At the bulk stage collect sample from the each pool of thirty MF III of Switzerland HP in a sterile tube.
(ii)Label the tube with the identification details of the corresponding container of the pooled extract and store the tubes in an ice-box (Temp. 2-8^0 C) and transfer the samples to laboratory.

3. **Final Batch Stage (Bulk) Sampling**

(i)At the bulk stage collect the in process samples of MF III of Switzerland HP Injectables in previously cleaned sterile glass tubes.
(ii)Label the tube with the identification details of the corresponding batch number of the in process MF III of Switzerland HP injectables and store the tubes in an ice-box (Temp. 2-8^0 C) and transfer the samples to laboratory.

ANNEXURE VII

QUALITY CONTROL DEPARTMENT

Document	**SPECIFICATION**
Product Name: MF III OF SWITZERLAND HP SOLUTION	
Generic Name : MF III OF SWITZERLAND HP SOLUTION	

Standards : In-House	Shelf Life : 7 Days

SERIAL NO.	PARAMETERS	SPECIFICATION
1.	DESCRIPTION	Colourless clear liquid.
2.	IDENTIFICATION	Chromatographic analysis of amino acid profile should be matching with the standard HPLC chromatogram
3.	pH	6.8 to 7.2
4.	HEAVY METAL	Not More Than 10 ppm
5.	ARSENIC	Not More Than 1 ppm
6.	SULPHATED ASH	Not More Than 0.1 %
7.	ASSAY Each mL contains	Total Nitrogen :Not More Than 0.08 % (w/v)
8.	HIV Antibody, HBS Antigen & HCV Antibody	Absent

Prepared By	Checked by	Approved By
Executive – QC	In-charge QC	Head QA

ANNEXURE VIII

METHOD OF ANALYSIS OF MF III OF SWITZERLAND HP SOLUTION

1. Identification :

The amino acid profile chromatogram of the test sample has to be comparable to that of Standard Sample of MF III of Switzerland HP Solution.

Method of amino acid profile development (By HPLC)

HPLC System (model : LC – 10A, make : Shimadzu)

1.1 The system employs derivatisation reaction of amino acids with OPA (ophthalaldehyde).
1.2 The system includes HPLC equipment as a main unit and can analyze free amino acids as well as hydrolyzed amino acids.

Stationary Phase

The system uses styrene-diviny1 benzene copolymer with sulfonic acid, i.e. strongly acidic cation exchange resin, (sodium type) for separation.

Detector

Fluorescence Detector (model : RF – 10AXL)

Reagents

xii) Sodium Citrate
xiii) n-Caprylic Acid
xiv) Ethanol 99.5%
xv) Perchloric Acid 60%
xvi) Sodium Carbonate
xvii) Potasium Sulphate
xviii) o-phthalaldehyde
xix) Brij-35
xx) Sodium Hypochlorite
xxi) 2-Mercaptoethanol
xxii) Sodium Hydroxide
xii) Distilled water HPLC grade

Mobile Phases

Chemical Composition :

	Liquid A	Liquid B	Liquid C
pH	3.20	10.00	pH not adjusted
Sodium Citrate $2H_2O$ (g)	58.8	58.8	-
Sodium Hydroxide (g)	-	-	4
Boric Acid (g)	-	12.4	-
Ethanol (99.5%) (ml)	210	-	-

Perchloric Acid (ml) 4N NaOH solution (ml)	50 -	- 30 ml	- -
Final volume (In liter)	3	1	0.5

Procedure for preparing 4N sodium Hydorxide aqueous solution for pH adjustments.

Weigh out 32g of NaOH and dissolve in 200ml of distilled water.
Conduct pH fine adjustment for the solutions A and B accurately using digital pH meter and standard buffer solutions.

Filtration Of Mobile Phase

Separately filter mobile phases A,B and C through 0.45 micron membrane filter. Take care so that the solutions do not mix with each other and that they are not contaminated with ammonia.

Preparation Of Derivatization Reaction Reagent :

Buffer

Chemical Composition	Quantity In Gms	Molar Concentration
Sodium Carbonate	122.2	0.384M
Boric Acid	40.7	0.216M
Potassium Sulphate	56.4	0.108M

Dissolve the above reagents in water to make 3 liter solution. Use this buffer without pH fine adjustment.

Brij-35, 10% solution (polyoxyethelene lauryl ether)

Weigh out 10g of Brij-35 to make 100 ml of solution. Put the bottle in warm water to facilitate dissolution of Brij-35.

Reaction Reagent A [Sodium hypochlorite solution (NaClo solution)]

Take 500 ml of boric acid-carbonic acid buffer by a measuring cylinder. And 0.2ml of Commercially available sodium hypochlorite aqueous (effective chlorine concentration around 7-10%) solution. Mix them thoroughly and filter through 0.45 micron membrane filter.

Reaction Reagent B (0.08% OPA solution)

Chemical Composition

Reagent	Quantity
OPA	400 mg
Ethanol	7 ml
2-mercaptoethanol	1 ml
10% Brij – 35 solution	2 ml
	Add buffer to make 500 ml of solution

Preparation Of Diluent For Sample

Weight out 9.8 g of sodium citrate. Add about 400ml of distilled water to dissolve the sodium citrate. Then, add 8ml of perchloric acid and 0.05 ml of n-caprylic acid. Add distilled water to make 500ml solution. Then, adjust the pH of the solution to 2.20 by adding perchloric acid.

Sample Solution

Use MF III of Switzerland HP Solution as it is.

Chromatographic System

A. Column

Separation column : Shimpack ISC-07 / S1504 Na (4mm ID × 15 cm long)
(Make : Shimadzu)
Guard column : Shimpack ISC-07 N a (4 mm ID × 15 cm long)
(Make : Shimadzu)
Ammonia trap column : Shimpack ISC-30 /S0504 Na(4 mm ID × 15 cm long)
(Make : Shimadzu)

B. Run Parameters

Total flow of mobile phase : 0.300 mL/ minute

Pressure Maximum : 100.0 kgf / cm^2

Pressure Minimum : 0.0 kgf / cm^2

Run Time : 90 minutes

Reaction Reagent Flow Rate : 0.2 mL / minute (each)

Column Oven Temperature : 55 °C

C. Detector Settings

Excitation wavelength : 348 nm

Emission wavelength : 450 nm

Spectrum type : Emission

Gain : x 4

Sensitivity : Low

Scan Speed : Fast

Sampling Period : 500 mili second

Delay : 0 minute

D. Gradient program

Time	Event	Value
0.01	B..Conc	0.0
7.00	T.Flow	0.3
12.00	T.Flow	0.1
17.00	B..Conc	0.0
24.00	T.Flow	0.1

24.01	T.Flow	0.2
29.00	T.Flow	0.3
35.00	B..Conc	16.0
40.00	B..Conc	16.0
40.01	B..Conc	60.0
45.01	T.Flow	0.3
50.00	B..Conc	100.0
52.00	T.Flow	0./3
52.02	T.Flow	0.1
55.00	T. Flow	0.1
56.00	T.Flow	0.3
58.00	T.Flow	0.3
60.00	B..Conc	100.0
60.01	B..Conc	0.0
60.02	SV (Pump A)	1.0
65.00	SV (Pump A)	0.0
90.00	STOP	0.0

2. pH

Take about 50 mL of sample in a 100 mL beaker. Immerse the electrode of the pH meter into the solution and measure the pH of the sample at 25°C ± 2°C

3. Heavy Metals

Prepare test solution and standard solution as directed below :

Standard solution :

Into a 50-mL Nessler cylinder pipette 1.0 mL of lead standard solution (20 ppm Pb) and dilute with water to 25 mL. Adjust with dilute acetic acid or dilute ammonia solution to a pH between 3.0 and 4.0, dilute with water to about 35 mL and mix.

Test solution :

Place 2.0 g of the sample into a 50-mL Nessler cylinder and make the volume to 25 mL with water. Adjust with dilute acetic acid or dilute ammonia solution to a pH between 3.0 and 4.0, dilute with water to about 35 mL and mix.

Procedure:

To each of the cylinders containing the standard solution and test solution respectively add 10 mL of freshly prepared hydrogen sulphide solution, mix, dilute to 50 mL with water, allow to stand for 5 minutes and view downwards over a white surface; the colour produced with the test solution is not more intense than that produced with the standard solution.

4. Arsenic

Into the bottle or conical flask introduce 10.0 g of the substance being examined; add 50 mL of water and 10 mL of stannated hydrochloric acid. Into the resulting solution add 5 mL of 1M potassium iodide and 10 g of zinc AsT.

Immediately assemble the arsenic apparatus and immerse the flask in a water bath at a temperature such that a uniform evolution of gas is maintained. After 45 minutes any stain produced on the mercuric chloride paper is not more intense than that obtained by treating in the same manner 1.0 mL of arsenic standard solution (10ppm As) dilute to 50 mL with water.

5. Sulphated Ash

Heat a silica crucible at 800°C for 10 minutes, allow to cool in a desiccator and weigh. Transfer to the crucible 1 g of the substance being examined and weigh the crucible and the contents accurately. Ignite, gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 mL of sulphuric acid, heat gently until the white fumes are no longer evolved and ignite in a muffle furnace, at a temperature $800^{O} \pm 25^{O}$ C, until all black particles have disappeared and continue heating until a white or at most greyish residue is produced. Allow to cool the crucible in a desiccator to room temperature and weigh again. Repeat the operation until two successive weighings do not differ by more than 0.5 mg. Calculate the percentage of sulphated ash using the following formula :

$$\text{Sulphated Ash} : \frac{[\text{Final weigh of crucible} - \text{Initial weigh of crucible}]}{\text{Weight of sample taken in g}} \times 100\ \%$$

6. ASSAY

Equipment Required

1. Kjeldalh flask 250 mL : 1 No.
2. Distillation set-up : 1 Set.
3. Glass funnel : 1 No.
3. Conical flask 250 mL : 1 No.
4. Standard burette 50 mL : 1 No.
5. Glass bead : 2 Nos.

Reagents Required

1. Potassium sulphate / sodium sulphate anhydrous, A.R.
2. Cupric sulphate, AR
3. Purified water
4. Sulphuric acid solution, 0.01N
5. Sodium Hydroxide solution 50% w/v
6. Boric Acid solution, 4% w/v
7. Methyl red - Mathylene blue indicator solution

Mix 10mL of methyl red solution (0.1% in alcohol) with 10mL of methylene blue solution (0.125%in alcohol).

Procedure

Transfer accurately 10 mL of MF III of Switzerland HP solution into a 250 mL kjeldahl flask. Add 2 gm of powdered potassium sulphate 200 mg of powdered cupric sulphate and 10 mL of sulphuric acid . Add two glass beads through the wall of kjeldahl flask at 45° in a fuming chamber. Place a funnel on the month of kjeldahl flask. Heat the mixture gently (at low flame), keeping the temperature below the boiling point until frothing has ceased. Increase the heat, until the acid boils briskly and continue the heating until the solution has become clear green in colour or almost colourless for 30 minutes.

Allow to cool, add 100 mL of purified water . Mix the contents of the flask and cool it again by keeping the flask in crushed ice. Make the distillation set ready by assembling different parts of distillation assembly. Add cautiously 50 mL of 50 % sodium hydroxide solution slowly in such a manner that the solution flows down the inner side of the kjeldahl flask to form a layer under the acid solution. Without delay, connect the flask to a kjeldahl connecting bulb(trap), previously attached to a condenser. The delivery tube from this condenser should be dipped into a mixture of 25 mL of 4 % boric acid solution and 75 mL of distilled water kept in a conical flask.

Heat the flask gradually for distillation until about four-fifth of the content of the flask has distilled over. Rinse the condenser with purified water and transfer all the rinsing solution to the conical flask. Add 3 to 4 drops of methyl red-methylene blue solution to the boric acid solution. Titrate with 0.01N sulphuric acid.

Each mL of 0.01N sulphuric acid is equivalent to 0.0001401 g of nitrogen.

$$\text{Calculation : Content of Total Nitrogen} = \frac{\text{Titre value} \times 0.0001401 \times F \times 100}{10} \%\ (w/v)$$

7. HIV ANTIBODY, HCV ANTIBODY AND HBS ANTIGEN

List of Accessories

1. ELISA Kit for detection of
   HIV-1 and HIV-2 Antibodies
   HCV Antibodies
   HBs Antigen

2. Distilled water
3. Micropipette of 100 µl and 1000 µl capacity and related disposable tips.
4. Microplate washing instrument
5. Measuring cylinder of 100 ml.
6. Disposable absorbent paper
7. Disposable gloves
8. Autoclavable 5 lit. beaker
9. Microplate ELISA reader

HBs antigen testing method

Bring all the reagents to room temperature before use except the color reagent. Store the color reagent at 2-8^{0}C till use. Then remove required number of microwells from the packet and label them appropriately. In each run use one reaction blank, three negative controls and one positive control.

Fill all the wells with 100 µl of sample diluent and add 100 µl of negative control, positive control and test solutions to the respective wells. Keep the first well as the reaction blank. Cover all the strips with adhesive strip cover after shaking. Incubate the strips for 1 hour at room temperature (25^{0}C – 40^{0}C).

After completion of the incubation time discard the adhesive strip cover and aspirate the contents of the wells. Then add 50 µl of conjugate stabilizer first, followed by 100 µl of conjugate to each well except blank. Then agitate all strips gently for 5- 10 seconds to mix the contents of the micro wells. After that incubate the strips at room temperature for 30 minutes.

After completion of the incubation decant the content of the wells into a waste container by the autowasher. Then fill all wells with diluted washing buffer (approx

350 µl) and soak for 30 seconds and again wash. Repeat the washing four times more. Finally remove excess fluid by tapping on a disposable absorbent paper.

Then add 100 µl of color reagent in each well and incubate for 30 minutes in dark at room temperature.

After that add 100 µl of stopping solutions in all wells to stop the reaction.

Finally take the absorbance reading bichromatically at 450 nm using 630 nm as reference wave length with the help of ELISA reader.

Calculation for determination of Cut off value

Cut off value is the mean OD of negative control (NCX) + 0.100

Interpretation of result

All the samples with the absorbance less than that of Cut off value should be considered negative and the absorbance is equal to or greater than that of Cut off value should be considered positive for HBs.

Note:
(i) The absorbance of negative control must be less than 0.100
(ii) The absorbance value of positive control must be greater than 1.500
(iii) The assay run is considered to be valid if the absorbance of the negative control, positive control are within the acceptable range. If they are not, technique or reagents should be suspected to be at fault and the assay should be repeated.

HIV 1&2 Antibody testing method

Bring all reagents and samples to room temperature except the colour reagent which is to be stored at 2-8^{0}C till use. Then remove required numbers of micro wells from the packet and label them appropriately. Keep the blank well empty. Use reaction blank, three negative and one positive control for each run**.**

Fill 200 µl of sample diluent in all of the wells except the reaction blank well. Then add 10 µl of negative, positive and test solution to the respective wells. Cover all the strips with adhesive strip cover after gentle shaking and incubate it for 30 minutes at room temperature (20^{0}C to 30^{0}C).

Discard the adhesive strip cover after the incubation period and decant all the contents of the wells into a waste container by auto washer. Fill the diluted washing buffer (approx 350 µl) and allow it to soak for 30 seconds. Then decant it in the waste container. Repeat the same for four more times and finally remove the excess fluid by tapping the strips on a disposable absorbent paper.

Add 50 µl of conjugate to each of the wells except the reaction blank well immediately after tapping. Cover the strips after gentle mixing of the contents of the microwells and incubate for 30 minutes at room temperature (20^{0}C -30^{0}C).

Discard the adhesive strip cover after the incubation period and wash five times by the diluted washing buffer as discussed above. Add 100 µl of colour reagent into each well including the reaction blank and keep 15 minutes in dark.
After that, add 100 µl of stopping solution in all the wells including the reaction blank control to stop the reaction.

Finally take the absorbance reading bichromatically at 450 nm by using 630 nm as reference wave length by the ELISA reader.

Calculation for determination of Cut off value

Cut off value is the mean OD of negative control (NCX) + 0.225

Interpretation of result

All the samples with the absorbance less than that of Cut off value should be considered negative for anti HIV and the absorbance is equal to or greater than that of Cut off value should be considered positive for anti HIV.

Note:
(i) The absorbence of individual negative control must be greater than - 0.010 O.D units &less than or equal to 0.200 O.D units. One negative control may be discarded if it is outside of this range. Then mean negative control (NCX) is calculated from the two remaining values instead of three negative control values.
(ii) The absorbance value of positive control should be atleast 1.000.
(iii) The absorbance value (O.D) of reagent blank should be between 0.000 to 0.100.
(iv)The assay run will be valid if the O.D values of positive control, negative control and reagent blank are within the acceptable range. If they are not, technique or reagents should be suspected to be at fault and the assay should be repeated.

HCV antibody testing method

Bring all the reagents in room temperature before use except colour reagent (to be stored at 2-8^0 C till use). Remove the required number of antigen coated microwells/ strips from the packet. Label the wells appropriately. Leave the reaction blank well empty. Use reaction blank, three negative and one positive control for each run.

Fill 200 µl of sample diluent in all of the wells except the reaction blank well. Then add 10 µl of negative, positive and test solution to the respective wells. Cover all the strips with adhesive strip cover after gentle shaking and incubate it for 30 minutes at room temperature (20^{0}C to 30^{0}C).

Discard the adhesive strip cover after the incubation period and decant all the contents of the wells into a waste container by auto washer. Fill the diluted washing buffer (approx 350 µl) and allow it to soak for 30 seconds. Then decant it in the waste container. Repeat the same for four more times and finally remove the excess fluid by tapping the strips on a disposable absorbent paper.

Add 50 µl of conjugate to each of the wells except the reaction blank well immediately after tapping. Cover the strips after gentle mixing of the contents of the microwells and incubate for 30 minutes at room temperature (20^0C -30^0C).

Discard the adhesive strip cover after the incubation period and wash five times by the diluted washing buffer as discussed above. Add 100 µl of colour reagent into each well including the reaction blank and keep 15 minutes in dark.

After that, add 100 µl of stopping solution in all the wells including the reaction blank control to stop the reaction.

Finally take the absorbance reading bichromatically at 450 nm by using 630 nm as reference wave length by the ELISA reader.

Calculation for determination of Cut off value

Cut off value is the mean OD of negative control (NCX) + 0.225

Interpretation of result

All the samples with the absorbance less than that of Cut off value should be considered non-reactive for anti HCV and the absorbance more than that of Cut off value should be considered reactive for anti HCV.

Note:

(i) The absorbance of individual negative control must be greater than 0.000 O.D units & less than or equal to 0.200 O.D units. One negative control may be discarded if it is outside of this range. Then mean negative control (NCX) is calculated from the two remaining values instead of three negative control values.

(ii) The absorbance value of positive control should be atleast 1.0.

(iii) The absorbance value (O.D) of reagent blank should be between 0.000 to 0.100.

(iv) The assay run will be valid if the O.D values of positive control, negative control and reagent blank are within the acceptable range. If they are not, technique or reagents should be suspected to be at fault and the assay should be repeated.

ANNEXURE IX

Product Name	**MF III OF SWITZELAND HP 230MG PLACENTA EXTRACT**		
Batch Number	C00104	COA Number	AR/COO104
Batch Size	240 Liter	Analysis Date	12.11.06
Manufacturing Date	11.11.06	Sampled Quantity	100 mL
Use Before	7 Days	Sampling Date	12.11.06

Test	**Specification**	**Result**
Description	Colourless clear solution.	Colourless clear solution
Identification	Chromatographic analysis of amino acid profile should be matching with the standard HPLC chromatogram	Complies
pH	6.8 to 7.2	6.9
Heavy Metal	Not more than 10 ppm	Less than 10 ppm
Arsenic	Not more than 1 ppm	Less than 1 ppm
Sulphated Ash	Not more than 0.1 %	0.02 %
Assay Total Nitrogen Content :	Not More Than 0.08 %(w/v)	0.011 % (w/v)
HIV Antibody, HBS Antigen & HCV Antibody	Absent	Absent

Product Name	**PLACENTRAE EXTRACT SOLUTION**		
Batch Number	C00204	COA Number	AR/C00204
Batch Size	240 Liter	Analysis Date	26.08.06
Manufacturing Date	26.08.06	Sampled Quantity	100 mL
Use Before	7 Days	Sampling Date	26.08.06

Test	**Specification**	**Result**
Description	Colourless clear solution.	Coloured clear solution
Identification	Chromatographic analysis of amino acid profile should be matching with the standard HPLC chromatogram	Complies
pH	6.8 to 7.2	7.0
Heavy Metal	Not more than 10 ppm	Less than 10 ppm
Arsenic	Not more than 1 ppm	Less than 1 ppm
Sulphated Ash	Not more than 0.1 %	0.03 %
Assay Total Nitrogen Content :	Not More Than 0.08 %(w/v)	0.014 % (w/v)
HIV Antibody, HBS Antigen & HCV Antibody	Absent	Absent

ANNEXURE X

PROCESSING

A) Collect 236.4 liters from the approved bulk of placenta extract.
Filter the solution of step (2) through 0.8 µ membrane filter fitted in 293 mm membrane holder (sterilised) and collect in sterilised SS 316 pressure vessel.
Check the integrity of the membrane filter by bubble point test.
Inform Quality Control to draw the sample for In process analysis.

B) FILLING & SEALING

1) Wash and sterilise 2 mL colourless ampoules as per SOP No. P-15.
2) Wash and sterilise filling machine parts and other accessories (tubing, needles, syringes etc) SOP No. P-08.
3) Set the filling machine for 2.15 mL dose and fill each ampoules from the approved bulk solution.

Fill volume range – 2.05 mL to 2.20 mL.

Seal the ampoules. Reject improper sealed ampoules.
Check the fill volume and clarity of filled ampoules every ½ hour and record in the BPR.

C) TERMINAL STERILISATION

Sterilise the filled ampoules in autoclave (Fabwell or Machine Fabrik) (SOP No. P-06 / P-07) at 121°C, 15 lbs/sq. inch for 20 minutes.

D) LEAK TEST

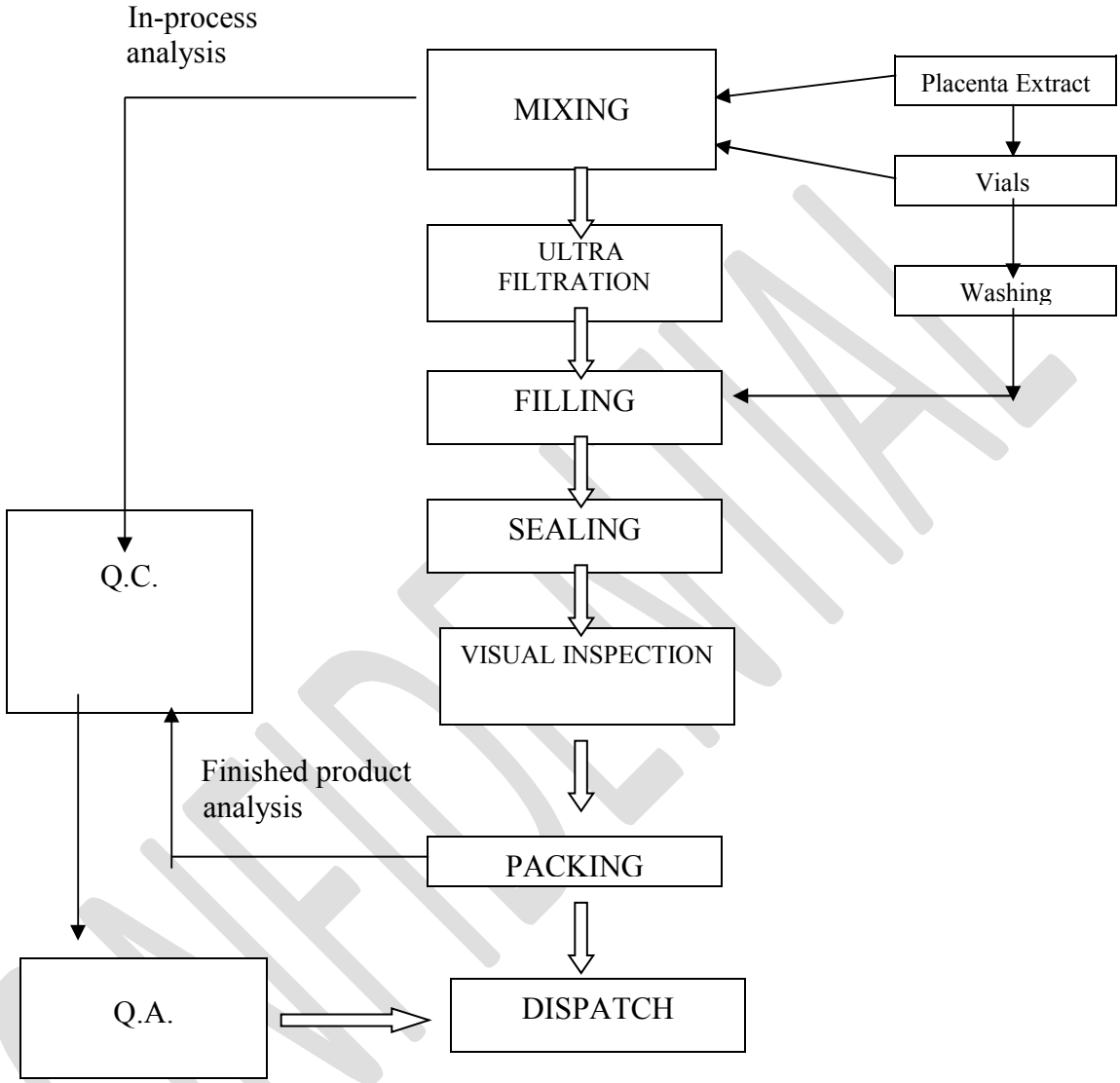
Carry out the leak test for any minute leakage in the ampoules by applying vacuum of 1 kg/cm^2. for successive three times. Then wash the ampoules with distilled water. Reject and destroy the defected ampoules.

E) INSPECTION

1) Inspect each and every ampoule as per SOP No. P-19 reject the ampoules showing, particulate matter, crack, less volume or any other physical defects. Record the quantity and type of reject.
2) Destroy the inspection-rejected ampoules as per SOP No. P-32 and record in the Batch Process Record.

ANNEXURE XI

MANUFACTURING PROCESS FLOW



ANNEXURE XII

RAW MATERIAL SPECIFICATION

ITEM	WATER FOR INJECTIONS		
MOLECULAR FORMULA	H_2O	MOLECULAR WEIGHT	18.02
ITEM CODE NO.	RRW03	SPECIFICATION NO.	RM/RRW03/01
STANDARD	IP + BP	EFFECTIVE	APRIL, 2006
PAGE	1 of 1	SUPERSEDES	RM/SPEC/03

NO.	TESTS	SPECIFICATIONS
1.	Description	A clear; colourless; odourless solution.
2.	pH	4.5 to 7.0
3.	Conductivity	NMT 1.1 µS cm^{-1}
4.	Acidity and Alkalinity	To comply the test.
5.	Chloride	To comply the test.
6.	Sulphate	To comply the test.
7.	Nitrate	NMT 0.2 PPM
8.	Ammonium	NMT 0.2 PPM
9.	Calcium and Magnesium	To comply the test.
10.	Heavy Metals	NMT 0.1 PPM
11.	Oxidisable Matter	To comply the test.
12.	Residue on Evaporation	NMT 0.001% w/w
13.	Bacterial Endotoxins	NMT 0.25 EU/ml

Prepared By	Checked By	Approved By
Executive – QC	In-Charge – QC	Head – QA

ANNEXURE XIII

METHOD OF ANALYSIS OF WATER FOR INJECTIONS

DESCRIPTION : A clear colourless liquid, odourless and tasteless liquid.

pH : Determine the pH of the sample with the help of a suitable pH meter.

CONDUCTIVITY : Determine the conductivity of the sample at 20^{O}C, with the help of a suitable conductivitymeter.

ACIDITY AND ALKALINITY : Take 50 ml of sample in a borosilicate flask and heat to boiling. Continue boiling for 2 minutes and allow to cool to room temperature.

To 10 ml of the sample add 0.05ml of methyl red solution, the resulting solution is not red.

To 10 ml of the sample add 0.1ml of bromothymol blue solution, the resulting solution is not blue.

AMMONIUM : Prepare test solution and standard solution as directed below :

Standard solution : Into a 50-ml Nessler cylinder pipette 4 ml of ammonium standard solution (1 ppm NH_4) and dilute to 20 ml with water.

Test solution : Into a 50-ml Nessler cylinder pipette 20 ml of the sample.

Procedure : To each cylinder containing the standard solution and the test solution respectively, add 1 ml of alkaline potassium tetraiodomercurate solution, diluted to 25 ml with water and allow to stand for 15 minutes. After 15 min, when viewed downwards over a white surface; the colour produced with the test solution is not more intense than that produced with the standard solution.

CALCIUM AND MAGNESIUM : To 100 ml of the sample add 2 ml of ammonia buffer pH 10, 50mg of mordant black mixture and 0.5 ml of 0.01M Disodium Edetate; a pure blue colour is produced.

CHLORIDE : To 10 ml of the sampleadd 1 ml of 2 M nitric acid and 0.2 ml of 0.1 M silver nitrate solution, the appearance of the solution does not change for at least 15 minutes.

SULPHATE : To 10 ml of the sampleadd 0.1 ml 2M Hydrochloric acid and 0.1 ml of barium chloride solution. The appearance of the solution does not change for 1 hour.

OXIDISABLE MATTER : To 100 ml of the sample add 10 ml of 1M sulphuric acid and 0.1 ml of 2.02 M potassium permanganate solution and boil for 5 minutes, the solution remains faintly pink.

RESIDUE ON EVAPORATION : Dry a suitable petridish at 105^{O}C for half an hour. Allow to cool in a desicator and weigh. Transfer 100.0 g of the sample and accurately weigh the petridish. Place the loaded petridish on a dry bath maintained at 105^{O}C. Heat the sample at 105^{0} C until all the sample get evaporated. Then collect the petridish place it in a dry heat oven maintained at 105^{O}C for half an hour. Remove the petridish and allow it to cool to room temperature in a desiccator before weighing. Weigh the petridish again. The residue weigh not more than 1 mg. Calculate the content of non volatile matter using the following formula :

$$\text{Non volatile matter} = \frac{[\text{Final weigh of petridish} - \text{Initial weigh of petridish}]}{\text{Weight of sample taken in g}} \times 100\ \%$$

HEAVY METALS : Prepare test solution and standard solution as directed below :

Standard solution : Into a 50-ml Nessler cylinder pipette 10.0 ml of lead standard solution (1 ppm Pb) and dilute with water to 25 ml.

Test solution : Evaporate 150 ml of the sample to 15 ml in a borosilicate evaporating vessel on a dry bath. Allow to cool to room temperature and pipette 12 ml of the sample into a 50-ml Nessler cylinder.

Procedure : To the cylinder containing the standard solution add 2.0 ml of the test solution and mix. To each of the cylinders containing the standard solution and test solution respectively add 2 ml of acetate buffer pH 3.5, mix, add 1.2 ml of thioacetamide reagent, allow to stand for 2 minutes and view downwards over a white surface; the colour produced with the test solution is not more intense than that produced with the standard solution.

NITRATE : Prepare test solution and standard solution as directed below :

Standard solution : Into a 20 ml test tube pipette 0.5 ml of nitrate standard solution (2 ppm NO_3) and dilute with water to 5 ml. Immersed the test tube in iced water for about 15 minutes.

Test solution : Into a 20 ml test tube pipette 5 ml of the sample and immersed the test tube in iced water for 15 minutes.

Procedure : To each test tubes containing the standard solution and the test solution respectively, add 0.4 ml of a 10% w/v solution of potassium chloride, 0.1 ml of diphenylamine solution and, dropwise with shaking, 5 ml of nitrogen-free sulphuric acid. Transfer the tubes to a water-bath at 50°C. After 15 min, when viewed downwards over a white surface, any blue colour produced with the test solution is not more intense than that produced with the standard solution.

BACTERIAL ENDOTOXIN TEST : Determine as per B.P..

Annexure XIV

STABILITY DATA

STABILITY STUDY DATA ON MF III OF SWITZERLAND HP INJECTION

Time	*Batch No.*	*Mfg. Date*	*Exp. Date*	*Analytical Parameters*				
				Description	*pH*	*Total Nitrogen*	*Sterility*	*Abnormal Toxicity*
Initial	*1P01030*	*March 2001*	*March 2007*	*Clear liquid*	*7.10*	*0.015 % (w/v)*	*Complies*	*Complies*
After 12 Months				*Clear liquid*	*6.99*	*0.015% (w/v)*	------	------
After 24 Months				*Clear liquid*	*6.97*	*0.015% (w/v)*	------	------
After 36 Months				*Clear liquid*	*6.99*	*0.015% (w/v)*	------	------
After 48 months				*Clear liquid*	*6.92*	*0.016% (w/v)*	*Complies*	*Complies*
Initial	*1P01070*	*November 2001*	*November 2007*	*Clear liquid*	*6.93*	*0.016% (w/v)*	------	------
After 12 Months				*Clear liquid*	*6.86*	*0.017% (w/v)*	------	------
After 24 Months				*Clear liquid*	*6.88*	*0.017% (w/v)*	------	------
After 36 Months				*Clear liquid*	*6.87*	*0.017% (w/v)*	------	------
After 48 months				*Clear liquid*	*6.90*	*0.016% (w/v)*	*Complies*	*Complies*
Initial	*2P01010*	*December 2001*	*December 2007*	*Clear liquid*	*6.20*	*0.014% (w/v)*	------	------
After 12 Months				*Clear liquid*	*6.28*	*0.014% (w/v)*	------	------
After 24 Months				*Clear liquid*	*6.28*	*0.014% (w/v)*	------	------
After 36 Months				*Clear liquid*	*6.30*	*0.014% (w/v)*	------	------
After 48 months				*Clear liquid*	*6.50*	*0.014% (w/v)*	*Complies*	*Complies*

Conclusion : The product is found to be stable during its assigned shelf life of 2 years and is safe for use during this period.

H-Nonclinical Data

Section (H)
Part III : Nonclinical Data (Safety) For Pharmaceutical Registration

Section A : Table Of Contents

SECTION D: NONCLINICAL STUDY REPORTS (IF APPLICABLE)
Table contents
Pharmacology
Pharmacokinetics
Toxicology
SECTION E: LIST OF KEY LITERATURE REFERENCES
Section B : Non Clinical Overview

1. General Aspects
Placenta is an immunologically privileged organ as is evident from the following facts :-

- Survival of semi allografts i.e. the embryo and allografts (graft rejection is very rare in pregnancy)
- Trophoblast layer of placenta is not antigenic
- Fibrinoid and mucopolysaccharide (sialomucin) covering on trophoblast cells mask surface antigens
- Suppression of cell mediated as well as humoral immunity by HCG and progestagens

The above immunological functions are possibly brought about by

a. Very small nucleotides, PDRN, Oligonucleotides, fragments of DNA, RNA
b. Smaller peptides
c. Amino acids
d. Water soluble growth promoting substances

The knowledge of MF III of Switzerland HP therapy dates back to Chinese civilization when it was used to treat a number of conditions ranging from wound healing to skin diseases.

Explorers confirmed 'Placentophagy' (Act of eating placenta) was prevalent in savage tribes. Even today it is prevalent in some tribals as well as animals.

The placenta plays a very active role during pregnancy and is essential for the well being of mother and child. Placenta produces a number of substances crucial for the development of foetus. These substances also help to maintain an immunological balance between mother and child. Medical scientists are more or less convinced now that placentae contains physiologically active substances.

From time to time placenta materials were the source of immunoglobulins. Some extract were used in various skin disorders eg. vitiligo, psoriasis, alopecia.

Dr. V P Filatov, a Russian professor popularized the use of placenta with his first publication in 1955. Filatov was an opthalmologist who successfully grafted human corneas.
In 1933 Filatov proposed doctrine of "Biogenic Stimulators". He observed that damage and stressed tissue developed a certain compound to survive.

In his literature review on " The Placentae" David Butlin mentions, placenta contains peptides similar to hypothalamic factors. This was further corroborated by Shibashaki et al in 1982 ……

In the same year Biachini et al reported the presence of "polydeoxyribonucleotide" a DNA nucleotide fraction in placenta , responsible for inactivating the complement component system, the kallikrein-kinin system along with suppression of hemocoagulation cascade, which when in excess causes hypersensitivity reaction.

Laboratoires Dom-AVMM Suisse worked on fresh human placenta containing many of the nutrients that pass from the material circulation to the fetal circulation.

The unique property of recruitment of immune system (due to the presence of the above ingredients in their most natural forms) has made placenta indispensable in abating inflammation and in aiding wound healing.

2. Content and structure format

Section C: Non Clinical Written and Tabulated Summaries

C1. Non-clinical Written Summary

1.1 Introduction

It is known that certain endogenous chemical mediators are released at the site of cellular injury and are responsible for the inflammatory reaction namely vasodilatation, leukocyte emigration and increased vascular permeability.

These factors are histamine, serotonin, prostaglandin, leucotrienes and lysosomal compounds.

The DNA nucleotide fraction in placenta is responsible for inactivating the complement component system, the kallikrein-kinin system along with suppression of hemocoagulation cascade, which when in excess causes hypersensitivity reaction.

In animal models of Hutchinsons plastic sponge induced inflammation and carageenin induced edema Palcentrex has shown significant local anti-inflammatory effect. (Biachini et al 1981).

Until recently extract of placenta has been shown to produce hypothalamic CRF like activity on the release and synthesis of ACTH & B endorphins. This indirectly acts via the HPA axis to inhibit arachidonic acid synthesis, thus inhibiting prostaglandins and leukotrienes formation. (Shibasakhi et al. 1982)
Beta-endorphin probably acts as a natural opioid in pain sensation, and analgesic property of MF III of Switzerland HP may be attributed to it.

CRF also acts as an important mediator of interaction between the nervous system and immune system.

Components of placenta possess immuno-modulatory activity**,** both humoral and cell mediated immunity are stimulated.

Humoral immunity is stimulated as is evident by increased levels of IgG and IgM (K U Ansari et al, 1991, 1994).

There is simultaneous insurgence of cell mediated immunity and an increase in number of lymphocytes specially T lymphocytes is observed. (K U Ansari 1994)

Control of infection in indolent leg ulcers with placenta has been faster that certain antibiotics when used alone – a fact that can be attributed to the elevated immune status (Humoral & cell mediated). (T Subramanian)

Wound healing and shortening of wound length may also be due to the immuno-stimulant properties.

Besides, amino acids, nucleotides and other nutrients present in MF III of Switzerland HP increase cellular biosynthesis favouring the anabolic phase.

Wound healing is further aided by CRF like activity of placenta, which may stimulate adenylate cyclase thus increasing the cAMP level and facilitating glycogenolysis leading to increased endogenous glucose as the source of energy. (J Endocrinol Metab 55: 384-86, 1982).

Keratinocyte growth promotion, thymidine incorporation studies were undertaken and a positive response was an indication of wound repair. Whether these effects were brought about by IL – 1 is still under study. (O'Keefe et al 1985, 1988). Growth induced by MF III of Switzerland HP was 50 times more potent than body's own EGF.

Certain nucleotides present in MF III of Switzerland HP have been directly related to the wound healing activities. Nucleotides promote growth factors, which are necessary for healing activity. Yet others promote healing by induction N O.

1.2 General presentation issues

C2: content of non-clinical written and tabulated summaries.

2.1 Pharmacology

2.1.1 Primary Pharmacodynamics

2.1.1.a **Anti-inflammatory activity.**

PDRN in placenta inhibit IgE mediated immediate type of hypersensitivity. This helps in anaphylactic type of reaction & allergies.

MF III of Switzerland HP also inhibits IgG mediated type III hypersensitivity. It suppresses excess of antigen antibody reaction and macrophage production, responsible for phagocytosis. (Bianchini et al).

An activity present in MF III of Switzerland HP has a strong CRF like function. This has a similar dose response on the release of ACTH & B endorphins. ACTH stimulates endogenous cortico-steroid formation, which exerts considerable anti-inflammatory effect. (Shibasaki et al).

2.1.1.b **Wound healing activity.**

Nucleotides present in the MF III of Switzerland HP promote the release of growth factors responsible for wound healing and repair.

MF III of Switzerland HP contain peptides that stabilized the enzyme trypsin and thus exerted the Wound healing activity.

Nucleotides present in the MF III of Switzerland HP promote the release of growth factors responsible for wound healing and repair. (G Tonello et al)

MF III of Switzerland HP exerts de-bridement action on necrotic tissue. This is essential for wound healing.

NADPH present in MF III of Switzerland HP stimulates the enzyme N O synthase to act upon L-arginine released from the injured tissue. This liberates N O, which helps in wound healing and repair, promotes angiogenesis collagen deposition etc.
(P Dutta et al)

Biochemical screening and mechanism of MF III of Switzerland HP

Biochemical analysis of MF III of Switzerland HP detected free amino acid on chromatography, very small peptides on gel electrophoresis and also nucleotides fragments on gel electrophoresis and spectroscopic analysis.
The presence of nucleotide fragments has been further confirmed by HPTLC studies. Small nucleotides with lower molecular weight were detected to be more predominant compared to peptides on spectroscopy. On TLC and subsequently HPLC identified 3-4 major and 1-2 minor components. One of these components, exhibited antibacterial as well as anti fungal properties.

Different batches of the drug were studied and batch wise variations were insignificant This is important so far as the standardization of the drug and its manufacturing is concerned

In vitro studies - Mechanism of Action of MF III of Switzerland HP

Spectrofluorometric analysis of MF III of Switzerland HP showed emission spectra identical to NADPH.
Presence of NADPH was further confirmed by HPLC and TLC with different solvents.
NADH & NADPH –has a big role in wound healing:

Normal human keratinocytes contain a constitutive enzyme N O synthase. This enzyme is cytosolic and requires the presence of Ca_{2+}, NADH, NADPH & FAD N O synthase activity is essential for wound repair and tissue regeneration of cutaneous wound.

It has also been reported that wound repair is characterized by sustained induction, activation and secretion by T Cells. Presence of functionally active N O synthase is a crucial prerequisite for normal wound re-epithelialisation. Proliferation of keratinocyte cell line is stimulated by presence of low concentration of N O donors.

N O is an active mediator important for host defence. It is produced by the L-arginine and NADPH dependent enzyme N O synthase.

In the acute phase of wound repair N O -

- Debridement action on necrotic tissue
- Promotes angiogenesis
- Causes cellular migration
- Increased collagen deposit
- Collagen cross linking
- Vasodilatation
- Increase viability of cutaneous flaps
- Helps to kill bacteria
- Enhance cellular immunomodulation & host defense locally

In chronic inflammation and repair, it probably stimulates the growth factors, which are NO dependent.

Maintain microcirculation

2.1.1.c **Immunotropic effect.**

MF III of Switzerland HP has stimulatory effect on both humoral immunity. This is evidenced by production of antibodies (immunoglobulins) from B-Lymphocytes.

MF III of Switzerland HP injection results in activation of T cells responsible for recognition of antigen and cell mediated immune response.

(K U Ansari et al)

2.1.2 Secondary Pharmacodynamics

An aqueous extract of human placenta possesses a number of interesting pharmacological effects in rats. It produces significant enhancement of wound healing. It also possesses a notable anti-inflammatory effect as tested by cotton pellets implantation method in rats.

The anti-inflammatory effect of MF III of Switzerland HP is in contrast to that of adreno-corticosteroids in the sense that it accelerates both the healing process & shortens wounds. It also has analgesic effect, which is statistically significant.

In partially hepatectomised rats, administration of MF III of Switzerland HP does not significantly affect the rate of regeneration. However, the mitotic index of the regenerating liver of the drug-treated animals is highly significant.

It does not produce any toxicity at the maximum dose levels, which can be administered to the different species of lab animals. The drug does not evoke any sensitization reactions elicited by antigen.

The results of general pharmacology may be summarized as: -

Motor activity

There was no difference between control and the drug treated animals as measured by Treamil and E.M.A. counter method in rats and mice.

Effects on smooth muscles

Rats Uterus: Administration of 1 ml /25 gm of MF III of Switzerland HP did not produce any effect by itself. 1 ml. of the drug partially blocks the contractile effect of 0.75 units of posterior pituitary extract.

Guineapig Ileum : MF III of Switzerland HP at a dose level of 2 ml. produced partial blockade of the contractile response induced by histamine and acetylcholine.

Rabbit Jejunum : Administration of MF III of Switzerland HP produces significant relaxation and partially blocked the contractile response of histamine and acetylcholine.

Effects on in vivo intestinal contraction of dogs

In anaesthetized dogs, MF III of Switzerland HP does not have any direct effect on the intestinal movement and did not modify the effects of other drugs, such as Noradrenaline, Adrenaline, Histamine etc.

Effects on in vivo uterine contractions

In anesthetized female dogs MF III of Switzerland HP does not have any direct effect on the uterine movement and does not modify the effects of other drugs such as posterior pituitary extract .

Effects on Cardiovascular System on Frog Heart (In Situ)

0.1 ml of MF III of Switzerland HP produced negative Chronotropic and inotropic effect on frog's heart. After a brief myocardial inhibition, which is transient, the rate returns to normal after a few minutes. It had no effect on the augmentation of force of contractions on heart rate produced by adrenaline and isoprenaline. The myocardial inhibitory effect of the drug was not blocked by Atropine.

Effects on Blood pressure or anaesthetized dog

Intravenous administration of MF III of Switzerland HP even at dose levels of 10 ml. does not produce any effect on Blood pressure, heart rate of respiration.

Analgesic effect by hot plate method

The MF III of Switzerland HP appears to possess significant analgesic effect when tested compared with control

The effect on healing wounds reveals that MF III of Switzerland HP has a statistically significant effect.

The anti-inflammatory studies of MF III of Switzerland HP reveals that percentage inhibitions of granuloma tissue when compared to control are statistically highly significant. The doses from 0.5 ml to 1 ml. produces maximum anti-inflammatory effect in the ex-periments conducted which was similar to that produced by 100 mg/kg phenylbutazone.

Sensitization studies :

Were conducted by administration of MF III of Switzerland HP in animals previously exposed to the drug. On the 15th, 30th and 45th day after the first injection no evidence of any sensitization reaction was visible thereby showing lack of antigenicity. Addition of MF III of Switzerland HP to the isolated organ bath in which was mounted the lluem of sensitized guinea pig, did not produce any contraction. The Dale Schuitz reaction is negative.

Protection against allergen induced Bronchospasm (Systemic Anaphylaxis)

Intravenous administration of the antigenic material dose of egg albumin produces bronchospasm in both control and MF III of Switzerland HP treated animals. Administration of egg albumin to the isolated bath containing smooth muscles strips of ileum of sensitized to MF III of Switzerland HP shows that it offers no protection against sensitization reactions to other antigens.

Effect on Liver regeneration

Body Weight: Liver Weight.

The body weight of the unoperated animals, partially hepatectomised control animals and partially hepatectomised MF III of Switzerland HP treated animals does not reveal any significant changes in weight of the liver between control and drug treated animals. So, total regeneration is not adversely affected.

Mitotic Index

There is a difference in mitotic index of the operated control rats and the operated MF III of Switzerland HP treated rats. The number of cells under mitosis in atleast 5 fields (H.P.) has been observed and the average of the control groups and treated groups have been compared. It has been found that there is a significant increase of actively dividing cells in the treated animals than in the untreated control groups. The effect is significant in all 5 groups.

2.1.3: Safety Pharmacology

There was no significant difference in the behavioral pattern between the control and drug treated animals. The drug when administered intraperitonealy at the maximum dose level of 1 ml/ 25 gm. In mice did not produce any effect. In rats even the maximum dose of 3ml/ 100 gm intraperitonealy did not produce any significant changes. All the animals withstood exhibiting symptoms of toxicity and there was no mortality.

2.1.4. Pharmacodynamics Drug Interactions

Not reported.

2.2. Pharmacokinetics

2.2.1. Absorption

2.2.2. Distribution

2.2.3. Metabolism (Inter-Species Comparison)

2.2.4. Excretion

It is a multiple component drug of natural origin constituents are peptides and nucleotides. These follow the normal pharmacokinetics of the body.

2.2.5. Pharmacokinetic Drug Interaction (Non-Clinical)

Not available

2.2.6. Other Pharmacokinetic Studies

Not available

2.3. Toxicology

2.3.1. Single dose toxicity

The drug when administered intraperitoneally at the maximum dose level of 1 ml/ 25 gm. In mice did not produce any toxic effect.

In rats even the maximum dose of 3ml/ 100 gm intraperitoneally did not produce any significant changes.

The LD 50 of the drug is much higher than average tolerable dose. The corresponding calculated human LD50 was 3.75 per kg and is several times higher than the recommended dose. (1 ml per day)

2.3.2. Repeat dose toxicity

MF III of Switzerland HP was administered at 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 ml per 20 gm of mice in intraperitoneal route. It was carried out continuously for 72 hours. It was observed that MF III of Switzerland HP was safe up to 45 ml/kg body weight in intraperitoneal route of mice.

2.3.3 Genotoxicity

None reported

2.3.4. Carcinogenicity

None reported

2.3.5. Reproductive and Developmental Toxicity

2.3.5.1. Fertility and Early Embryonic Development

2.3.5.2. Embryo-Fetal Development

2.3.5.3. Pre-Natal and Post Natal Development Including Maternal Function

2.3.6. Local Tolerance

None reported.

2.3.7. Other Toxicity Studies, If available

Not applicable.

C3. Non-clinical tabulated summaries

Sl No.	Author	Subject	Journal
1	T Shibasaki et al	Corticotropin-Releasing Factor like activity in placenta	Journal of Clinical Endocrinology & Metabolism 1982; 55 (2) 384-86
2	P Bianchini et al	Pharmacological Data on Polydeoxiribonucleo-tide of human placenta	Int. J Tiss. Reac. III (3-4) 151 -154 (1981)
3	O'Keefe et al	Keratinocyte Growth-promoting activity from human placenta	Journal of cellular Physiology 124, 439-445, 1985
4	O"Keefe et al	Stimulation of Thymidine Incorporation in Keratinocytes by Insulin, Epidermal Growth Factor, and MF III of Switzerland HP : Comparison with Cell Number to Assess Growth	J Invest Dermatol 90 : 2-7, 1988
5	K U Ansari et al	Immunomodulating Potential of Certain Agents	Jr of Assn. of Phys. of India, Jan' 91
6	K U Ansari et al	An experimental and clinical evaluation of immuno-modulating potential of placenta	Indian Journal of Pharmacology 1994; 26 : 130 - 132
7	Giuseppe Tonello et al	Characterization and quantitation of the active polynucleotide fraction (PDRN) from human placenta, a tissue repair stimulating agent	Journal of Pharmaceutical and Biomedical Analysis 14 (1996) 1555- 1560
8	T K Biswas et al	Wound healing activity of MF III of Switzerland HPs in rats	Acta Pharmacologica Sinica 22 (12) : 1057 - 1180 : Dec 2001
9	T K Sur et al	Anti-inflammatory and anti-platelet aggregation activity of placenta	Acta Pharmacologica Sinica 24 (2) : 187 - 192 : Feb 2003
10	L Kameshwaran	Pharmacological study on placenta	Data on file

Section D: Non-clinical study reports
(if applicable)

Table contents

Pharmacology

Pharmacokinetcs

Toxicology

Not applicable.

Section E : List of key literature reference

1. Immunomodulating Potential of Certain Agents : MF III of Switzerland HP Injection
K.U. Ansari et al, Deptt. of Pharmacology, M.L.N. Medical College, Allahabad, U. P. India: Report published in Journal of Association of Physicians of India, January 1991.

2. Corticotrophin- Releasing Factor-like Activity in MF III of Switzerland HPs
Tomotsu Shibasaki et al Jr. of Clinical Endocrinology & Metabolism 1982; 55(2) 384 – 86.

3. Pharmacological Data On Polydeoxiribonucleotide of Human Placenta
Bianchini et al Int. J. Tiss. Reac. III(3.4) 151-154 (1981)

4. Keratinocyte Growth-Promoting Activity from Human Placenta
E.J.O'Keefe et al Jr. of Cellular Physiology 124-439-445, 1985.

5. Stimulation of Thymidine Incorporation in Keratinocytes by Insulin, Epidermal Growth Factor, and MF III of Switzerland HP : Comparison with Cell Number to Assess Growth
Edward J. O'Keefe et al Dept. of Dermatology, University of North Carolina, USA.

6. Anti-inflammatory and anti-platelet aggregation activity of MF III of Switzerland HP
Sur T K et al Acta Pharmacol Sin 2003 Feb ; 24 (2) : 187-192

7. Characterization and quantitation of the active polynucleotide fraction (PDRN) from human placenta, a tissue repair stimulating agent
Giudeppe Tonello et al Jr. of Pharmaceutical and Biochemical Analysis 14(1996) 1555-1560.

8. An experimental and clinical evaluation of immuno-modulating potential of placenta
K.U. Ansari et al Indian Jr. of Pharmacology 1994; 26:130-132.

9. Wound healing activity of placenta in rats
Biswas Tuhin Kanti et al Acta Pharmacological Sin 2001 Dec; 22 (12); 1113 – 1116

10. Pharmacological Study on placenta
Dr. Lalitha Kameswaran et al M.G.r. Medical University , Madras.

I-clinical data

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Part IV: Clinical Document

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7. Case Reports Forms and Individual Patient Listing

8. Publish clinical papers

Section F. List of key Literature References

Section B. Clinical overview

1. Product Development Rationale

Placenta was in use as medicine since very old times. Wound healing and anti-inflammatory properties made it one of the unique medicines in tissues healing and recovery of wound. In the modern scenario antibiotics and surgery (wherever necessary) are the main stay of therapy in wound management. As such there is very little scope of drug therapy in tissue regeneration and wound management. MF III of Switzerland HP offers an excellent scope of drug therapy in tissue repair.

Injection of placenta has also proven role in pelvic inflammatory diseases like salphingitis and infertility due to tubal inflammation.

2. Overview of Biopharmaceutics

3. Overview of Clinical Pharmacology

MF III of Switzerland HP is active in many chronic inflammatory conditions. This along with its wound healing activities has been reported in various international clinical reports. The drug has been used in Gynaecology in conditions like post radiation cervicovaginitis, dystrophic and chronic cervicovaginitis . It is proposed that MF III of Switzerland HP may be active against some of the mechanisms involved in chronic inflammation.
Local application of MF III of Switzerland HP favourably influence tissue formation and wound healing. MF III of Switzerland HP is particularly helpful in treating burn wounds, ulcers including diabetic ulcers as well as chronic non –healing wounds.

Local application of MF III of Switzerland HP favorably influence tissue formation and wound healing.

The drug also exhibited immuno-modulatory activity.

4. Overview of Efficacy

Placenta has been successfully used in chronic inflammatory conditions in Obstetrics & Gynaecology like salphingitis and other PIDs , infertility in tubal inflammation, cervical erosion, post operative cuts and episiotomies.

Placenta is highly effective in tissue regeneration, neoangiogenesis and epithelialisation. It has been successfully used in post surgical wounds radiation ulcers bedsores, chronic indolent ulcers, diabetic ulcers etc.

5. Overview of Safety

The LD50 of the drug is much higher than average tolerable dose. The corresponding calculated human LD50 was 3.75 per kg and is several times higher than the recommended dose. (1 ml per day)

The drug is very safe when administered within the recommended dose and may be used continuously for a prolonged period.

No adverse effect has been noted or reported so far.

6. Benefits and Risks Conclusions

The benefits of the drug have been proved beyond doubt. It has been in the market for a prolonged time. It being a derivative of a natural product the side effect and risks are minimum.

Section C. Clinical Summary

1. Summary of Biopharmaceutics Studies and Associated Method

Background and Overview

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Appendix 1.

2. Summary of Clinical Pharmacology Studies

2.1. Background and Overview.

MF III of Switzerland HP is active in many chronic inflammatory conditions. This along with its wound healing activities has been reported in various international clinical reports. The drug has been used in Gynaecology in conditions like post radiation cervicovaginitis, dystrophic and chronic cervicovaginitis . It is proposed that MF III of Switzerland HP may be active against some of the mechanisms involved in chronic inflammation. MF III of Switzerland HP is particularly helpful in treating burn wounds, ulcers including diabetic ulcers as well as chronic non –healing wounds

Local application of MF III of Switzerland HP favorably influence tissue formation and wound healing.

The drug also exhibited immuno-modulatory activity.

2.2. Summary of Results of Individual studies.

1)

· **MF III OF SWITZERLAND HP IN BURNS & WOUNDS**

MF III of Switzerland HP treated groups took 10 days to heal completely whereas control group with smaller size wounds took more than 12 days showing obvious advantage of MF III of Switzerland HP.

· **MF III OF SWITZERLAND HP ON NON HEALING ULCERS**

MF III of Switzerland HP was successful in healing these ulcers when all sorts of treatment failed.

· **MF III OF SWITZERLAND HP ON GROSSLY INFECTED WOUNDS**

Satisfactory healing was observed in these wounds with systemic MF III of Switzerland HP was observed in these wounds with systemic MF III of Switzerland HP .

· **PLACENTA ON BED SORES**

Placenta enhances the rate of healing and contraction of bedsores.

REF: ROLE OF PLACENTA IN INFECTED BURNS AND WOUNDS – DR. D.S. SUKLA , DEPT OF SURGERY , G.S.B.M. MEDICAL COLLEGE KANPUR.

2)

Placenta offers several advantages over the routinely used antibiotics and anti-inflammatory agents. This includes better antibacterial activity, vascularisation of wound environment and early fistula healing.

In chronic cases,

It reduces oedema and inflammation by corticotropin releasing factor like activity.

Elevation of immunoglobulins induced by MF III of Switzerland HP limit the spread of superadded bacterial infection.

Therefore the nutritional intake of patient improves and he takes more protein diet required to heal the fistula fast.

REF; ROLE OF MF III OF SWITZERLAND HP ON HEALING OF INTESTINAL FISTULAE, DR. M BANSALI

3)

MF III of Switzerland HP was applied topically over the wound. Dressing was changed daily. Patients were followed up till the wound healed.

Eleven (52%) patients had 75–100 % epithelialisation of the wound in a 4-8 week period. No patient experienced adverse reaction with the drug.

The present study shows that MF III of Switzerland HP has a beneficial effect in the management of chronic non-healing wounds.

2.3. Comparison and Analyses of Results Across Studies

Not available

2.4. Special Studies

K U Ansari et al

" Immuno-modulating Potential of certain agents" Journal of Association of Physicians of India, January 1991.

" An Experimental and clinical evaluation of immuno-modulating potential of placenta" Indian Journal of Pharmacology 1994:26 : 130-132.

Appendix 2

3. Summary of Clinical Efficacy

3.1. Background and overview of clinical efficacy

After 15 years of research MF III of Switzerland HP emerged as a biogenous stimulator based on the doctrine of Prof. V P Filatov, a Russian Ophthalmologist experienced in corneal rafting.

Corneal ulcer cases were treated with MF III of Switzerland HP successfully.

3.2. Summary of results of individual studies

1. B N Purandare et al

Placental therapy in tubal block

Placenta injections showed excellent result in cases of primary and secondary infertility and tubal blockade. 75% patients got their tubes opened.

The injection therapy with placenta deserves a trial in tubal block before subjecting the patient to major abdominal surgeries.

Paper presented in All India Obs. & Gynae Congress
published in "The Clinician" volume XXXIV, No. 1, 45-48, Jan, 1970

2. S Pati et al

Clinical Evaluation of Effect of Dressing with placenta in the treatment of infected wounds.

Infected wounds arising as a sequlae after different operation and bed sores of eclamtic mother were treated with local application of placenta; control of equal numbers were treated with povidone iodine and hydrogen peroxide.

Placental Injections was found to be very much effective, inexpensive and excellent stimulant of granulation tissue, moreover superior to the dressing of povidone iodine.

It promotes healing, acts as immunomodulating and has tissue regeneration activity. It produced dramatic improvement of healing of bedsores.

Placenta in the treatment of-infected wounds Jr. of Obs. & Gynae of India Vol. 51: No. 3; 124–126, 2001

3 Rashmi Kaul et al

Local application of MF III of Switzerland HP is an economical and safe drug, which can be used with impunity without any major side effects. It can be used wherever regeneration, anti-inflammatory, anti-edematous effect is required to give benefit to patient. Patients completed treatment of radiation schedule without interruption and reaction subsided with use of placenta thus increasing compliance of patient and providing good quality of life.
To evaluate the effect of local placenta therapy in reducing side effects of radiation in patients of cervical carcinoma, The Antiseptic, Vol. 98 No. 4 2001

4 Kaushal V et al

To evaluate placenta therapy in the treatment of radiation mucosities involving the oral / oropharyngeal region, a prospective randomized study was carries out in 120 patients with squamous cell carcinoma of the head and neck from August 1997 to March 1999. The study was conducted in patients receiving radicals external radiation therapy, planned for => 60 Gy/30F/6 weeks, who developed grade 2 radiation mucositis (patchy mucositis) during radiation treatment. The patients were randomized in two groups of 60 patients each to receive either placental treatment or conventional treatment (control group). Placental treatment was given as injection of 2ml by deep intramuscular injection 5 days a week for 15 injections. Conventional treatment given in the control group was dispirin gargles and betamethasone oral drops., A subjective decrease in pain was observed in 48/60 (80 %) of patients in placental therapy group compared with 22/60 (36.7%) in the control group. The progression to grade 3 radiation mucositis was 24/60 (40%) in the placental therapy group compared with 52/60 (86.7 %) in the control group. The subjective improvement in swallowing was seen in 56/60 (93%) of patients in the placental therapy group compared with 9/60 (15%) of patients in the control group. Only one patient in the placental therapy group compared with three in the control group required interruption of radiation therapy because of severe radiation reactions. MF III of Switzerland HP appears to be effective in the management of radiation – induced oral/ oropharyngeal mucositis and especially in controlling subjective symptoms.

Clinical evaluation of placental therapy in radiation induced oral mucositis

INT.J.TISSUE REACT XXIII(3) 105-110 2001

5 Chauhan & V K Shukla et al

Chronic wounds of the lower extremity are a therapeutic dilemma. In India, chronic wounds are caused by factors other than impaired circulation and diabetes, which account for most of the clinical problem in Western societies. A study of 2 topical agents, placenta and phenytoin powder is presented in the paper. One hundred fifty patients were randomly assigned to these treatments or to saline dressings (control). It was observed that patients receiving active topical treatments responded better than those in the control group. The importance of this finding should be viewed with the perspective that these topical treatments are inexpensive and easily available in India. The study also piloted measurements of angiogenic responses in 1 group, and the findings encourage further exploration with the technique and topical agent.
Eleven (52%) patients had 75-100% epithelialisation of the wound in a 4-8 week period. No patient experienced adverse reaction with the drug.

Non-healing Wounds – A Therapeutic dilemma,
The International Journal of Lower Extremely Wounds,
Vol. 2, Issue 1, March 2003

3.3 Comparison and analysis of results across studies

Not done

3.4. Analysis of clinical information relevant to dosing recommendations

Pelvic Inflammatory Disease

Injection MF III of Switzerland HP has been used once (2 ml) daily or on alternate days or as directed by the physician.

Wound healing

Injection MF III of Switzerland HP has been recommended once (2 ml) daily or on alternate days for 25 to 20 days or as directed by the physician.

A more frequent dose did not produce any significant advantage.

3.5. Persistent of efficacy and or tolerance effects

There is a consistency in the efficacy of the drug No tolerance phenomenon has been reported.

Appendix 3.

4. Summary of clinical efficacy

4.1. Exposure to drug

The drug is safe and there is no antigenicity.

4.2. Adverse events

The drug is devoid of any serious side effects when administered within the recommended dose.

4.3. Clinical Laboratory Evaluations

No adverse event reported.

4.4. Vital science, physical findings, and other observations related to safety

General Appearance – Normal.
Respiratory system – Not affected.
Cardio-vascular system - Normal
Liver and Kidneys – Normal
Skin – No rash or allergies.

4.5. Safety in special groups and situations

Data not available

4.6. Post Marketing Data

No unto word incidence reported.

Appendix 4.

5. Synopsis of individual studies.

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Paper presented in All India Obs. & Gynae Congress
published in "The Clinician" volume XXXIV, No. 1, 45-48, Jan,1970.

Placental injection therapy in tubal block

Placenta injections showed excellent result in cases of primary and secondary infertility and tubal blockade. 75% patients got their tubes opened.

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Local application of placenta is an economical and safe drug, which can be used with impunity without any major side effects. It can be used wherever regeneration, anti-inflammatory, anti-edematous effect is required to give benefit to patient. Patients completed treatment of radiation schedule without interruption and reaction subsided with use of placenta thus increasing compliance of patient and providing good quality of life.

4. Kaushal V et al

Clinical evaluation of placental therapy in radiation induced oral mucositis.
INT.J.TISSUE REACT XXIII(3) 105-110 2001

To evaluate placental therapy in the treatment of radiation mucosities involving the oral / oropharyngeal region, a prospective randomized study was carries out in 120 patients with squamous cell carcinoma of the head and neck from August 1997 to

March 1999. The study was conducted in patients receiving radicals external radiation therapy, planned for => 60 Gy/30F/6 weeks, who developed grade 2 radiation mucositis (patchy mucositis) during radiation treatment. The patients were randomized in two groups of 60 patients each to receive either Placental Therapy treatment or conventional treatment (control group). Placental Therapy was given as injections of 2ml by deep intramuscular injection 5 days a week for 15 injections. Conventional treatment given in the control group was dispirin gargles and betamethasone oral drops., A subjective decrease in pain was observed in 48/60 (80 %) of patients in placental therapy group compared with 22/60 (36.7%) in the control group. The progression to grade 3 radiation mucositis was 24/60 (40%) in the Placental Therapy group compared with 52/60 (86.7 %) in the control group.

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5. Chauhan & V K Shukla et al
Non-healing Wounds – A Therapeutic dilemma,
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Chronic wounds of the lower extremity are a therapeutic dilemma. In India, chronic wounds are caused by factors other than impaired circulation and diabetes, which account for most of the clinical problem in Western societies. A study of 2 topical agents, Placenta injections and phenytoin powder is presented in the paper. One hundred fifty patients were randomly assigned to these treatments or to saline dressings (control). It was observed that patients receiving active topical treatments responded better than those in the control group. The study also piloted measurements of angiogenic responses in 1 group, and the findings encourage further exploration with the technique and topical agent.

Eleven (52%) patients had 75-100% epithelialisation of the wound in a 4-8 week period. No patient experienced adverse reaction with the drug.

Section D. Tabular Listing of all clinical studies

Sl No.	Author	Subject	Journal
1	R Kaul et al	To evaluate the effect of local placental therapy in reducing side effects of radiation in patients of cervical carcinoma	The Antiseptic Vol 98 No. 4
2	S Pati et al	Clinical Evaluation of Effect of Dressing with placental injections in the Treatment of Infected Wounds	Obs. & Gyn of India Vol.51 No.3 : May/June 2002
3	B Purandare et al	injection therapy in tubal block	No.1, pages 45-48 : January 1970. Clinician, Vol: XXXIV
4	Kaushal V et al	Clinical Evaluation of placental injections in radiation-induced oral mucositis	Int J Tissue React. XXIII (3) 105 -110 (2001)
5	Chauhan V S et al	Non-healing Wounds - A Therapeutic Dilemma	The Int. Jr of Lower Extremity Wounds 2 (1); 2003 pp 40 - 45

Section E : Clinical study reports (if applicable)

Not applicable.

Section F : List of some key literature references

1. Placental Extract Injection Therapy in Tubal Block
B. N. Purandare et al , The Clinical, January 1970 Vol. XXXIV, No.1, Pages 45-48.

2. Clinical Evaluation of Effect of Dressing with placental therapy in the Treatment of Infected Wounds
S. Pati et al Dept. of Obst & Gyn & Dept. of Microbiology, B. S. Medical College, Bankura, W.B.

3. To evaluate the effect of local placental therapy in reducing side effects of radiation in patients of cervical carcinoma
Rashmi Kaul et al , THE ANTISEPTIC Vol. 98, No.4

4. Non healing Wounds --- A Therapeutic Dilemma
V S Chauhan et al, Lower Extremity Wounds 2(1); 2003 pp. 40-45

5. Clinical Evaluation of placental injections in radiation-induced oral mucositis
Kaushal V et al, Int. J. Tissue React. XXIII (3) 105-110 (2001)