



Guidance Manual

Quality Control Testing of Human Albumin

NATIONAL INSTITUTE OF BIOLOGICALS
(Ministry of Health & Family Welfare)
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I hope this document will provide guidance to manufacturers / importers of blood products to understand various policies and procedures followed by the institutue in pre-release certification of the batches submitted for evaluation. The document also gives an insight on the relevance of the quality control tests in clinical scenario which will be relevant for the medical professionals.

The guidance and support received from all the members who contributed was vital for the success. I am grateful for their constant support and help.

Any suggestion in improving the document is highly appreciable.

I wish all success who has contributed in bringing the guidance document.

Dr. J. P. Prasad
Head
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ABBREVIATIONS USED

AMC	Annual maintenance contact
BP	British Pharmacopoeia
CDSCO	Central Drugs Standards and Control Organization
CoA	Certificate of analysis
COP	Colloidal Oncotic Pressure
CHMP	Committee for Medicinal Products For Human Use
DCA	Drugs and Cosmetic Act
DCG(I)	Drugs Controller General of India
DID	Double immune diffusion
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C Virus
HIV	Human Immuno deficiency virus
HPLC	High performance liquid chromatography
HSA	Human Serum Albumin
IAEC	Institutional Animal Ethics Committee
IEC	International electro technical commission
IP	Indian Pharmacopoeia
IPC	Indian Pharmacopoeia Commission
IRS	International reference standard
ISO	International organization for standardization
NABL	National Accreditation Board for Testing and Calibration Laboratories
NCL	National control laboratory
NIB	National Institute of Biologicals
NIBSC	National Institute of Biological Standards and Control
QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure
SPC	Summary Product Characteristics
SR & RDU	Sample Receipt and Report Dispatch Unit
TTI	Transfusion transmitted infection

1.0 PURPOSE

This Guidance document is meant to provide assistance to Biopharmaceutical industry and health care professionals to understand various procedures followed by NIB regarding the quality control testing of human albumin and to facilitate compliance with the policies and procedures of the institute. Guidance document also provide assistance to staff and academicians in the related field to have an insight on the structural and functional aspects of this plasma derived proteins. The clinical significance of various tests recommended by the Indian pharmacopoeia to define the quality standards of these products are also described in this document. The document is based on various SOPs followed by NIB and the information obtained from international guidelines and publications.

2.0 SCOPE

This guidance document applies to the importers/manufacturers of human albumin and the regulatory authorities to enhance their understanding on the requirements for submission of batches and quality control testing of these products for pre-release certification. The document also applies to academicians and medical students to have an insight on the laboratory & animal tests recommended by the pharmacopoeia and their impact on the quality of these products.

3.0 INTRODUCTION – HUMAN PLASMA DERIVED PRODUCTS

Human Plasma for Fractionation is the starting material for manufacture of a range of medicinal products used for the treatment of a variety of life-threatening injuries and diseases. Although the therapeutic use of blood transfusion goes back to the beginning of the 20th century, it was not until the 1940s that the technique of plasma fractionation, devised by Cohn and colleagues, enabled the widespread use of medicinal products extracted from human plasma.

Improvements in protein purification and molecular separation technology have made available a wide variety of products, with medical applications covering a large field, and the therapeutic value of these is unquestioned. However, the potential for viral transmission is well recognised, and because of the large number of donations which are pooled, a single contaminated batch of a plasma-derived

product, with the contamination possibly originating from a single donation, can transmit viral disease to a large number of recipients. The recognition in the mid-1980's that plasma-derived medicinal products, in particular coagulation factor concentrates, had caused widespread transmission of human immunodeficiency virus (HIV) and hepatitis C (previously identified as non-A non-B hepatitis) resulted in major changes to the manufacturing processes, with the introduction of specific steps to inactivate or remove these and other blood-borne viruses. Infectious non-enveloped viruses were detected in certain plasma-derived medicinal products during the 1990's and early 2000's. Therefore, recent process development has been devoted to further reducing non-enveloped viruses such as hepatitis A (HAV) and parvovirus B19 (B19V).

Measures taken to prevent infection include selection of donors, screening of individual donations and plasma pools for markers of infection with known viruses and validation of the production process for inactivation or removal of viruses. From the 1990's on, measures designed to minimise contamination of the starting plasma have been improved by the refinement of serological test kits and the use of nucleic acid amplification technology (NAT) for the testing of viral DNA and RNA, thereby shortening the window period during which infectious donations are not detected.

Cases of apparent iatrogenic variant Creutzfeldt-Jakob disease (vCJD) infection by blood transfusion in man in the UK provide strong evidence that vCJD is transmissible through blood transfusion.

The safety of products manufactured from plasma, is dependent on the measures taken to minimise the contamination of the starting material (donor selection, screening and testing). Safety is enhanced by the application of virus inactivation procedures and technology that removes or reduces the level of blood-borne viruses and other infectious agents. Plasma derived medicinal products are inherently variable due to their biological nature, and the biological methods used to test them. Because of the complexity and variability, a high level of expertise is required for the regulation and batch release of these products.

4.0 HUMAN ALBUMIN

Human albumin is a major product of the fractionation of human plasma, with a long history of clinical use. While the manufacturing process of human albumin solutions has not undergone significant changes, these products have benefited from progress in pharmaceutical technology, quality control and quality assurance, which enhance their clinical safety. Besides the strict requirements of pharmacopoeia monographs which describe the minimal quality criteria with which products must comply, the quality of the final products relies on a careful in-process control, to guarantee the applicability of validated manufacturing processes and to prevent risks of chemical, bacteriological, viral or endotoxic contamination of the product

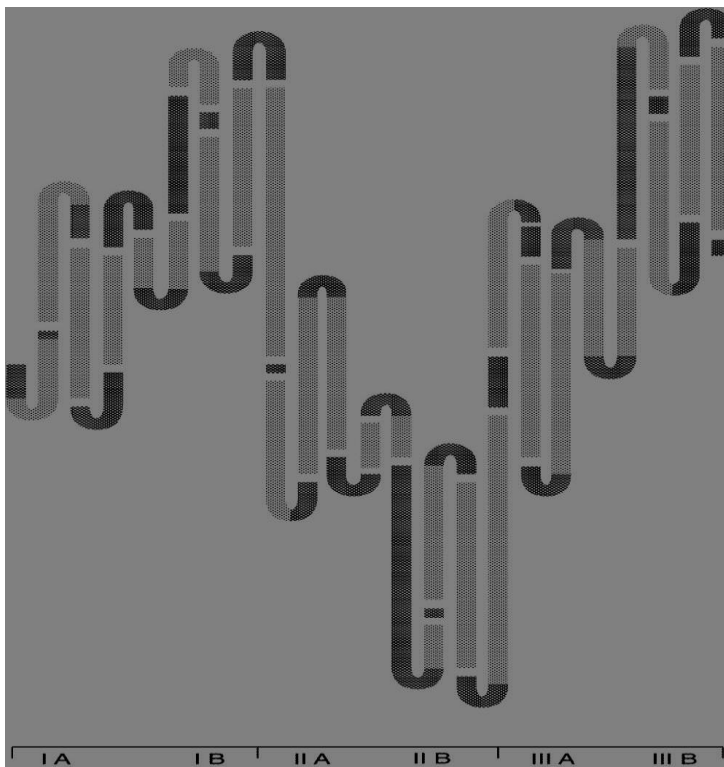
Albumin has a long history of clinical use in colloid replacement therapy dating back over 50 yr. It is currently used in greater volume than any other biopharmaceutical solution that is available, and worldwide manufacturing is of the order of 100s of tonnes annually. However, as with many therapies, the clinical use of albumin has often had its critics. When assessing the place for albumin in critical care therapy, the nature of the product being infused should be considered. Less pure preparations, such as plasma protein fraction, have been replaced by purer preparations with lower associated adverse reactions. Many of the earlier studies of albumin use were conducted in the 1970s and 1980s. Since that time, human albumin solution has been refined by developments and improvements to manufacturing processes, so that modern albumin is far removed from the solutions infused in earlier decades. Albumin solution for therapy should be as near as possible to the native protein found in the plasma, given the need for purification and viral assurance.

4.1 HUMAN ALBUMIN- STRUCTURE

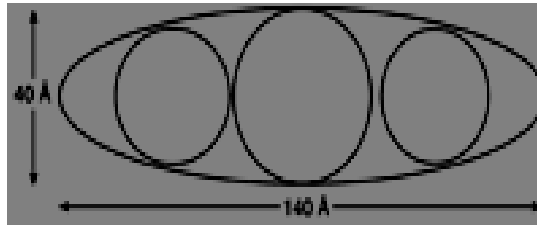
Albumin is a highly water-soluble protein (molecular weight 66 000 Da) with considerable structural stability. It is an important component of plasma, making up 60% of the total protein. At normal physiological concentrations of plasma proteins, albumin contributes 80% of the colloidal osmotic (oncotic) pressure of the plasma,

and its function as a carrier for hormones, enzymes, fatty acids, metal ions and medicinal products is much reported.

It consists of a single polypeptide chain of 585 amino acids. The chain is characterized by having no carbohydrate moiety, a scarcity of tryptophan and methionine residues, and an abundance of charged residues, such as lysine, arginine, glutamic acid and aspartic acid. The mature, circulating molecule is arranged in a series of α -helices, folded and held by 17 disulphide bridges. The folding creates subdomains of three contiguous α -helices in parallel (Fig. 1). A pair of subdomains face each other to form domains. These can be seen as cylindrical structures with polar outer walls and a hydrophobic central core.



The tertiary structure of human albumin crystal has been isolated by x-ray crystallography. It is seen as a heart-shaped molecule $80 \times 30 \text{ \AA}$.³⁶ In solution, the shape is quite different. The three domains appear to be arranged in an ellipsoid pattern, giving the molecule low viscosity (Fig. 2).



The ellipsoid structure of albumin in solution (British Journal of Anaesthesia 2000; 85: 599 -610)

The molecule is very flexible and changes shape readily with variations in environmental conditions and with binding of ligands. Despite this, albumin has a resilient structure and will regain shape easily, owing to the disulphide bridges, which provide strength, especially in physiological conditions. After their rupture, the molecule can re-establish these bridges and regain its structure. Denaturation occurs only with dramatic and non-physiological changes in temperature, pH and the ionic or chemical environment.

4.2 IN VIVO SYNTHESIS OF HUMAN ALBUMIN

In humans, albumin synthesis takes place only in the liver. Albumin is not stored by the liver but is secreted into the portal circulation as soon as it is manufactured. In healthy young adults, the rate of synthesis is 194 (sd 37) mg kg⁻¹ day⁻¹, or about 12–25 g of albumin per day. The rate of synthesis rate varies with nutritional and disease states. The liver can increase albumin synthesis to only 2–2.7 times normal because most of the liver's synthetic machinery is already devoted to albumin at rest. The synthetic pathway is common to eukaryotes and is also used for synthesis of other proteins.

Albumin will be synthesized only in a suitable nutritional, hormonal and osmotic environment. The colloid osmotic pressure (COP) of the interstitial fluid bathing the hepatocyte is the most important regulator of albumin synthesis.

Synthesis requires:

- mRNA for translation;
- an adequate supply of amino acids, activated by binding to tRNA;
- ribosomal machinery for assembly;
- energy in the form of ATP and/or GTP.

4.3 INVIVO DEGRADATION OF HUMAN ALBUMIN

Total daily albumin degradation in a 70 kg adult is around 14 g day^{-1} or 5% of daily whole-body protein turnover. Albumin is broken down in most organs of the body. Muscle and skin break down 40–60% of a dose of labelled albumin.¹⁰⁸ The liver, despite its high rate of protein metabolism, degrades 15% or less of the total. The kidneys are responsible for about 10%, while another 10% leaks through the stomach wall into the gastrointestinal tract.

4.4 FUNCTIONS OF ALBUMIN

The molecular structure of albumin has three main characteristics which may be considered important for critically ill patients: (i) cysteine residues, (ii) domains I and II, and (iii) imidazole residues. Cysteine residues in position 34 expose a –SH radical group (thiol), which is one of the main extracellular antioxidants. From this point of view, the administration of albumin to a critically ill patient during an acute pathological process usually increases the plasma concentration of thiols. Moreover, –SH residues bind nitric oxide to form S-nitrous thiols, thereby neutralising one of the most important mediators of pathological conditions such as sepsis. Albumin domains I and II are responsible for the transport of the numerous molecules, both endogenous and exogenous, that are extensively carried by human albumin. In this regard, it is evident how albumin concentration may be important when administering drugs with a high-binding affinity, especially during acute pathological processes usually characterised by hypoalbuminaemia. In these conditions, drug toxicity or even drug inefficiency may be observed. Finally, albumin has 16 histidine imidazole residues, which are responsible for the buffer function of albumin. In fact, having a pH of about 6.75, the residues may both give up or accept H^+ from the environment depending on the surrounding pH, thereby acting as a buffer molecule.

4.4.1 Oncotic pressure

In healthy subjects, the rollIn health, albumin contributes up to 80% of the normal COP of about 25 mmHg. This is because of its high molecular weight and concentration in plasma. Albumin is present at a higher concentration than other

plasma proteins, and though its molecular weight of 66.5 kDa is less than the average for serum globulins (about 147 kDa), it still has the greatest osmotic significance. This direct osmotic effect provides 60% of the oncotic pressure of albumin. The remaining 40% is a result of its negative charge, providing an attractive force for the intravascular retention of positively charged solute particles (the Gibbs–Donnan effect). Due to the large extravascular pool of albumin, its water-solubility and its negative charge, albumin also plays a significant role in the regulation of tissue fluid distribution.

4.4.2 Binding of substances to albumin

The structure of the albumin molecule is such that it can incorporate many different substances. It is a flexible molecule, and bound compounds can be buried within the structure. Some general trends have emerged from binding studies. Most strongly bound are medium-sized hydrophobic organic anions, including long-chain fatty acids, bilirubin and haematin. Less hydrophobic and smaller substances can be bound specifically but with lower affinity, such as ascorbate and tryptophan. The chirality of the compound may be important: l-tryptophan is bound more strongly than d-tryptophan. Monovalent cations do not bind, but divalent cations do, namely calcium and magnesium. Albumin has a strong negative charge, but there is little correlation between the charge of the compound and the degree of binding to albumin. Acidic drugs tend to bind to other plasma proteins such as α 1-acid glycoprotein whereas basic drugs tend to bind to albumin. There are exceptions, and drugs may bind to both.

Other endogenous compounds that bind to albumin include bile acids, eicosanoids, copper, zinc, folate and aquacobalamin. Albumin is also a secondary or tertiary carrier for some substances that have specific binding proteins, for example, steroids, including derivatives such as vitamin D and thyroxine. This can be clinically significant.

Other factors that are important in drug–albumin interactions and may be responsible for the wide interindividual variation seen include age (binding may decrease at the extremes); temperature, pH and ionic strength, which can affect the number of binding sites *in vitro*; and competition between drugs for binding sites.

There are a variety of binding sites on the albumin molecule. Sudlow *et al.*⁹⁸ have classified drugs into two groups according to two broad binding sites, site I and site II. Site I appears to lie along the long loop of subdomain IIA, extending into the shorter loop.⁷⁵ Many different drugs seem to bind here, including salicylates, warfarin, phenylbutazone, indometacin, digitoxin, furosemide, phenytoin, chlorpropamide and some penicillins. Dyes such as sulfobromophthalein, iophenoxate (a radio-opaque dye), methyl red, Evans blue and bromocresol green also bind here, as do endogenous compounds such as bilirubin.

4.4.3 Metabolic function

Apart from its vital role in transporting drugs and endogenous compounds, albumin is also involved in the inactivation of a small group of compounds. Disulfiram is inactivated by binding with albumin. Members of the penem group of antibiotics bind irreversibly to albumin, through acetylation of an ϵ -lysine group close to the surface of the molecule in the region of Sudlow site 1. The resulting complex may be clinically significant. Penicillin allergy has been linked to irreversible coupling of penicilloyl groups to these lysine groups. Coupling causes 'bisalbuminaemia', seen as a more rapidly moving albumin on an electrophoretic strip. This is associated with the appearance of antibodies to the drug–albumin complex (antipenicilloyl antibodies) in patients treated with penicillin.

Albumin is also involved in the metabolism of endogenous substances such as lipids and eicosanoids, because of the avidity with which these compounds bind to albumin.

4.4.4. Acid–base function

The presence of many charged residues on the albumin molecule and the relative abundance of albumin in plasma mean that it can act as an effective plasma buffer. At physiological pH, albumin has a net charge of negative. It is responsible for about half of the normal anion gap. A reduction in plasma protein concentration causes metabolic alkalosis. A decrease in serum albumin of 1 g dl^{-1} may increase standard bicarbonate by $3.4 \text{ mmol litre}^{-1}$, produce a base excess of $3.7 \text{ mmol litre}^{-1}$ and reduce the anion gap by $3 \text{ mmol litre}^{-1}$.

4.4.5 Antioxidant function

Under physiological conditions, albumin may have significant antioxidant potential. It is involved in the scavenging of oxygen free radicals, which have been implicated in the pathogenesis of inflammatory diseases. Physiological solutions of human serum albumin have been shown to inhibit the production of oxygen free radicals by polymorphonuclear leukocytes. This may be related to the abundance of sulfhydryl (-SH) groups on the albumin molecule. These are important scavengers of oxidizing agents, such as hypochlorous acid (HOCl) formed from the enzyme myeloperoxidase, which is released by activated neutrophil

4.4.6 Maintaining microvascular integrity

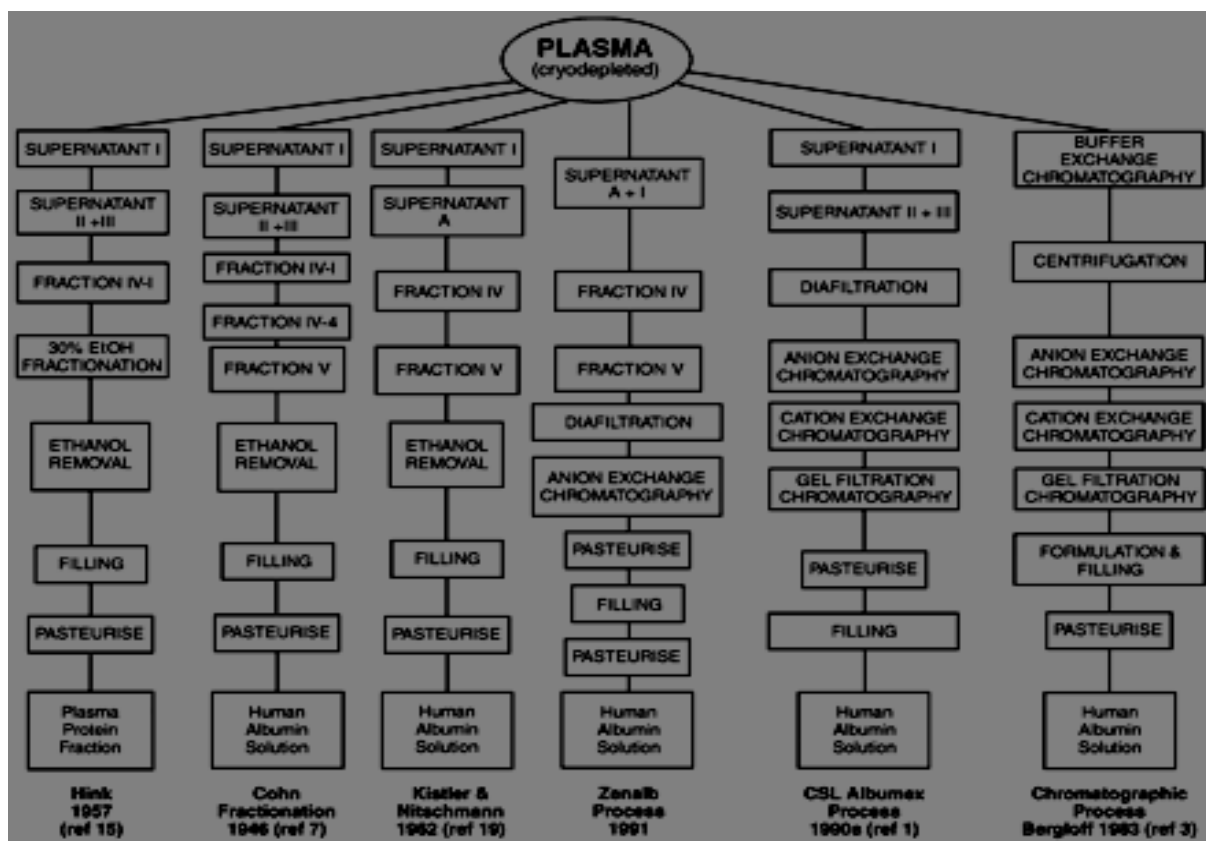
It is possible that albumin has a role in limiting the leakage from capillary beds during stress-induced increases in capillary permeability. Endothelial cells seem to be able to control the permeability properties of the capillary membrane, possibly by altering the nature and distribution of glycoproteins in the vessel wall. Albumin plays a part in this action, though the exact mechanism is not clear. It may involve the strong negative charge on the albumin molecule repelling other negatively charged molecules in the membrane, or it may be a space-occupying function of the albumin molecule that reduces the size of channels. It is likely that only a small amount of albumin is necessary for this function.

4.4.7 Anticoagulant effects

Albumin has effects on blood coagulation. It seems to exert a heparin-like action, perhaps related to a similarity in the structures of the two molecules. Heparin has negatively charged sulphate groups that bind to positively charged groups on antithrombin III, thus exerting an anticoagulant effect. Serum albumin has many negatively charged groups. There is a negative correlation between albumin concentration and the heparin requirement in patients undergoing haemodialysis.⁴² These investigations have shown a heparin-like activity of albumin, through enhancement of the neutralization of factor Xa by antithrombin III

4.5 MANUFACTURING OF HUMAN ALBUMIN SOLUTIONS

The traditional method for the purification of albumin for therapeutic use has been cold ethanol fractionation, as described by Cohn and colleagues in 1946 and its later variants. Since then, some pharmaceutical providers have chosen to supplement this process with additional purification steps while others have moved towards an alternative, predominantly chromatographic separation method (Fig 3).



4.6 INDICATIONS :

Some examples of the clinical guidelines for use of Human albumin are given below :
 {(4.6.1 - Clinical guidelines for human albumin use – developed by National Plasma

Product Expert Advisory Group (NPPEAG) Scotland [approved by NPPEAG, 14th December 2009]; 4.6.2 - Guideline on the core SPC for Human Albumin Solution (CPMP/PHVWP/BPWG/2231/99 rev.2) committee for medicinal products for human use : adoption by the CHMP 17th November 2005, Date for coming into effect 1 June 2006}}

4.6.1 CLINICAL GUIDELINES FOR HUMAN ALBUMIN USE – DEVELOPED BY NATIONAL PLASMA PRODUCT EXPERT ADVISORY GROUP (NPPEAG) SCOTLAND [APPROVED BY NPPEAG 14 DECEMBER 2009]

1. Ascites and large volume paracentesis:

Where management of ascites is **refractory** to sodium restriction (90mmol/day) HAS is indicated following paracentesis. Paracentesis without albumin replacement leads to a fall in pulmonary capillary wedge pressure, maximal at 6 hours, and can result in circulatory and renal dysfunction.

Where there is **normal** pre-morbid renal function:

- Administer **1 unit (100ml) HAS 20%** (STAT) following every **3 litres** of ascites drained.

Where renal function is **impaired** consider either:

- Administration of **100ml HAS 20%** per **2 litres** of ascites

or

adherence to the protocol for hepatorenal syndrome (see below)

2. Spontaneous bacterial peritonitis (SBP):

Administration of HAS in the setting of SBP reduces the incidence of renal failure and reduces mortality. HAS is indicated where there is a **rise in serum creatinine** in the setting of SBP:

- **Day 1: 1.5g HAS / kg** given over a 6 hour period:
- **Day 3: 1g HAS / kg** given over 3 hours

3. Hepatorenal syndrome (HRS)

Administration of HAS and vasoconstrictors are effective therapy in 60% of patients with HRS and is associated with improved survival

- **Terlipressin: 0.5 – 2mg iv every 4 hours, plus**
- **Day 1: 1g / kg HAS**
- **Day 2 - 16: 20 – 40 g HAS / day**

Rx continued until serum creatinine falls below 130mol/l

NB. Where creatinine is **rising** despite Rx, **60g HAS /day** may be clinically indicated

4. Therapeutic apheresis (Therapeutic Plasma Exchange TPE)

TPE has a clearly defined role in a large variety of conditions that are presumed to be immunologically mediated. The indications for TPE are protean and listed in a recent guideline produced by the American Society for Apheresis (Szczepiorkowski et al 2007). The standard replacement fluid for TPE is 5% albumin with or without 5% saline. FFP /Octaplas may be indicated dependent on the indication ie. TTP. The volume treated per procedure is usually: 1–1.5 total plasma volumes (TPV). Procedures may continue daily for up to several weeks in some cases.

5. Other indications

There is **little evidence** to support the use of HAS in other circumstances and these require discussion with haematology medical staff before release can be sanctioned.

Volume Expansion – Synthetic alternatives or Saline (5%) may be used for temporary intravascular volume expansion. No studies have shown that 4.5% Albumin confers any survival advantage.

6. Requesting Human Albumin Solution:

Human Albumin Solution (HAS) is available from the Hospital Pharmacy. The indication for its use should be documented in the case notes and there should be written documentation of its prescription.

7. Administration: 20% Albumin is hyperoncotic. The 100 ml volume will expand up to approximately 400 mls within 25 minutes of transfusion. Rapid administration can lead to rapid volume expansion and cardiac failure. It should be infused slowly to avoid this.

4.6.2. GUIDELINE ON THE CORE SPC FOR HUMAN ALBUMIN SOLUTION

**(CPMP/PhVWP/BPWG/2231/99 rev.2) COMMITTEE FOR MEDICINAL PRODUCTS
FOR HUMAN USE ; ADOPTION BY THE CHMP 17 November 2005; DATE FOR
COMING INTO EFFECT 1 June 2006**

CLINICAL PARTICULARS

1. Therapeutic indications

Restoration and maintenance of circulating blood volume where volume deficiency has been demonstrated, and use of a colloid is appropriate. The choice of albumin rather than artificial colloid will depend on the clinical situation of the individual patient, based on official recommendations.

2. Posology and method of administration

The concentration of the albumin preparation, dosage and the infusion-rate should be adjusted to the patient's individual requirements.

3. Posology

The dose required depends on the size of the patient, the severity of trauma or illness and on continuing fluid and protein losses. Measures of adequacy of circulating volume and not plasma albumin levels should be used to determine the dose required. If human albumin is to be administered, haemodynamic performance should be monitored regularly; this may include:

- arterial blood pressure and pulse rate
- central venous pressure
- pulmonary artery wedge pressure
- urine output
- electrolyte
- haematocrit/haemoglobin

4. Method of administration

Human albumin can be directly administered by the intravenous route, or it can also be diluted in an isotonic solution (e.g. 5 % glucose or 0.9 % sodium chloride) .The infusion rate should be adjusted according to the individual circumstances and the indication. In plasma exchange the infusion-rate should be adjusted to the rate of removal.

CPMP/PhVWP/BPWG/2231/99 rev.2 ©EMEA 2005 7/10

4. Contraindications

Hypersensitivity to albumin preparations or to any of the excipients.

6. Special warnings and special precautions for use

Suspicion of allergic or anaphylactic type reactions requires immediate discontinuation of the injection. In case of shock, standard medical treatment for shock should be implemented. Albumin should be used with caution in conditions where hypervolaemia and its consequences or haemodilution could represent a special risk for the patient. Examples of such conditions are:

- Decompensated cardiac insufficiency
- Hypertension
- Oesophageal varices
- Pulmonary oedema
- Haemorrhagic diathesis
- Severe anaemia
- Renal and post-renal anuria

The colloid-osmotic effect of human albumin 200 or 250 g/l is approximately four times that of blood plasma. Therefore, when concentrated albumin is administered, care must be taken to assure adequate hydration of the patient. Patients should be monitored carefully to guard against circulatory overload and hyperhydration. 200-250g/l Human albumin solutions are relatively low in electrolytes compared to the 40-50 g/l human albumin solutions. When albumin is given, the electrolyte status of the patient should be monitored and appropriate steps taken to restore or maintain the electrolyte balance. Albumin solutions must not be diluted with water for injections as this may cause haemolysis in recipients.

If comparatively large volumes are to be replaced, controls of coagulation and haematocrit are necessary. Care must be taken to ensure adequate substitution of other blood constituents (coagulation factors, electrolytes, platelets and erythrocytes). Hypervolaemia may occur if the dosage and rate of infusion are not adjusted to the patients circulatory situation. At the first clinical signs of cardiovascular overload (headache, dyspnoea, jugular vein congestion), or increased blood pressure, raised venous pressure and pulmonary oedema, the infusion is to be stopped immediately.

7 Interactions with other medicinal products and other forms of interactions

No specific interactions of human albumin with other medicinal products are known.

8. Pregnancy and lactation

The safety of {(trade) name of the product} for use in human pregnancy has not been established in controlled clinical trials. However, clinical experience with albumin suggests that no harmful effects on the course of pregnancy, or on the foetus and the neonate are to be expected.

9. Effect on ability to drive and use machines

No effects on ability to drive and use machines have been observed.

10 Undesirable Effects

Mild reactions such as flush, urticaria, fever, and nausea occur rarely. These reactions normally

disappear rapidly when the infusion rate is slowed down or the infusion is stopped.

Very rarely, severe reactions such as shock may occur. In these cases, the infusion should be stopped and an appropriate treatment should be initiated.

11. Overdose

Hypervolaemia may occur if the dosage and rate of infusion are too high. At the first clinical signs of cardiovascular overload (headache, dyspnoea, jugular vein congestion), or increased blood pressure, raised central venous pressure and pulmonary oedema, the infusion should be stopped immediately and the patient's haemodynamic parameters carefully monitored.

Further Reading - CPMP/PhVWP/BPWG/2231/99 rev.2 ©EMEA 2005 8/10

4.7 PHARMACEUTICAL FORM

Solution for infusion.

A clear, slightly viscous liquid; it is almost colourless, yellow, amber or green.

Human albumin is produced at two concentrations. The 4–5% albumin solution is an isotonic solution particularly suitable for fluid replacement in hypovolaemia. The 20–25% albumin is a hypotonic but hyperoncotic solution for the treatment of fluid loss where electrolyte or fluid load is contraindicated. The highly concentrated protein solution provides colloidal pressure while minimizing the additional salts and fluid volume that are infused. These low-salt, high-concentration albumin products are

also used to treat patients with poor renal function, to avoid electrolyte disturbances, and in the treatment of neonates.

4.8 PHARMACOPOEIAL STANDARDS FOR ALBUMIN

Human albumin has been used as a therapeutic agent for over 50 yr. Its key indication is the restoration and maintenance of circulating blood volume in situations such as trauma, surgery and blood loss, burns management and plasma exchange. The ideal product for this purpose would be monomeric albumin of very high purity, free from contamination with other plasma proteins, endotoxins, metal ions, albumin aggregates and prekallikrein activator (PKA), as such impurities appear to influence the tolerability of albumin infusion.

Experience with albumin products of the older generation suggests that high endotoxin concentrations may be implicated in febrile reactions, while high concentrations of PKA can cause hypotension. Aluminium concentrations need to be kept very low to avoid accumulation in neonates and patients with impaired renal function. Contamination with trace proteins may result in undesirable aggregation when albumin is being pasteurized. The goal for manufacturers in recent decades has therefore been to minimize or eliminate such impurities.

4.9 TESTS AS PER IP 2010 / BP 2012- HUMAN ALBUMIN SOLUTION

4.9.1 Description (Characters) :

A clear, slightly viscous liquid, it is almost colourless to greenish yellow or amber, depending on protein concentration and the method of fractionation used.

4.9.2 Identification:

A. Immunoelectrophoresis technique: Examine by a suitable immunoelectrophoresis technique. Using antiserum to normal human serum, compare normal human serum and preparation under examination, both diluted to contain 1.0 per cent w/v of protein. The main component of the preparation under examination corresponds to the Albumin component of normal human serum.

B. Precipitation test with a suitable range of species specific antisera which gives positive results for the presence of protein of human origin and negative results with antisera specific to plasma protein for other species.

4.9.3 pH

Dilute the preparation to be examined with a 9 g/L solution of *sodium chloride R* to produce a solution containing 10 g/L of protein; pH of the resulting solution 6.7 – 7.3.

4.9.4 Haem content:

Dilute the preparation to be examined using a 9 g/L solution of *sodium chloride* to obtain a solution containing 10 g/L of protein. The absorbance of resulting solution shall be not more than 0.15 . when measured by spectrophotometer at 403 nm.

4.9.5 Molecular Size Distribution: Liquid Chromatography (BP 2012)

The area of the peak due to polymers and aggregate is not greater 10% of the total area of the chromatogram.

4.9.6 Protein Composition: Zone electrophoresis

In the electropherogram obtained with the test solution on *cellulose acetate* or on *agarose gel*, not more than 5% of the protein has a mobility different from that of the principal band.

4.9.7 Stability : the contents of the final container remain unchanged, as determined by visual inspectiuon, after heating at 57oC for 50 hours, when compared to its control consisiting of a sample from the same lot which has not under gone this heating.

4.9.8 Pyrogen: Complies with the test for pyrogen using 3 mL/kg of the rabbit weight irrespective of the protein content, in rabbit that have not received blood products”

4.9.9 Sterility: Complies with the tests for sterility.

4.9.10 Abnormal Toxicity

Complies with the test for abnormal toxicity, using method B and 0.5 ml of the solution for each mouse and 5 ml for each guinea pig irrespective of the protein content.

4.9.11 Assay For protein

Human albumin contains not less than 95.0 % and not more than 105.0 % of the stated amount of protein.

4.9.12 For Sodium:

Dilute to 0.01 % w/v of protein with water and determine by atomic absorption spectrophotometry, or by flame photometry, measuring at about 589 nm and using *sodium solution FP* suitably diluted with *water* as the standard solution. The preparation under examination should contain not less than 95% and not more than 105% of the contents of Na stated on the label which is in any case not more than 160 millimoles of Na per litre. .

4.9.13 For potassium:

Dilute To 0.25 % w/v of protein with *water* and determine by atomic absorption spectrophotometry, or by flame photometry, measuring at about 767 nm and The preparation under examination should contain not less than 95% and not more than 105% of the contents of K stated on the label which is in any case not more than 2 millimoles of K per litre.

Human albumin intended for administration to patients undergoing dialysis or to premature infants complies with the following additional test.

4.9.14 Aluminium. Not more than 200 µg of Al per liter. Determine by atomic absorption spectrophotometry, with a furnace as a atomic generator and measuring at 309.3nm and using as standard solutions a suitable range of dilutions in water of *aluminium standard solution* (10ppm Al) *RS*.

4.10 Clinical significance of the tests recommended by India Pharmacopoeia

4.10.1 Test for pH

The presence of many charged residues on the albumin molecule and the relative abundance of albumin in plasma mean that it can act as an effective plasma buffer. At physiological pH, albumin has a net charge of negative. It is responsible for about half of the normal anion gap. A reduction in plasma protein concentration causes metabolic alkalosis. A decrease in serum albumin of 1 g dl^{-1} may increase standard bicarbonate by $3.4 \text{ mmol litre}^{-1}$, produce a base excess of $3.7 \text{ mmol litre}^{-1}$ and reduce the anion gap by $3 \text{ mmol litre}^{-1}$. Albumin has 16 histidine imidazole residues, which are responsible for the buffer function of albumin. In fact, having a pH of about 6.75, the residues may both give up or accept H^+ from the environment depending on the surrounding pH, thereby acting as a buffer molecule.

4.10.2 Test for identity

This is one of the important test as it confirms the origin as well as the identity of required plasma protein. This test is done by immunoelectrophoresis method using antiserum to human normal serum, and comparing normal human serum and the preparation under examination. The Indian pharmacopoeia 2010 recommends that the main component of the preparation to be examined correspond to the albumin component of normal human serum by immunoelectrophoresis.

The precipitation test in the Indian Pharmacopoeia is for confirming that the product is of human origin. For example to confirm that the product consists of human albumin the optimally diluted sample is allowed to diffuse in agar medium against the antisera developed in different animal species ie, Anti-bovine whole serum, Anti-horse whole serum, Anti goat whole serum, anti- rabbit whole serum as well as anti-human sera. The confirmation that product is of human origin and contains human albumin is shown by the precipitation band only against anti-human albumin. Precipitation against antisera from other species indicates adulteration of the product with

plasma from other species. This test assures the closest similarity of the components in the preparation to the required human protein for therapy.

4.10.3 Test for Haem content

Human serum albumin (HSA), the most prominent protein in plasma, is best known for its exceptional ligand (e.g., heme and drugs) binding capacity., ferric heme binding to HSA in the absence and presence of drugs has been investigated. Values of the association equilibrium constant for drug binding to Sudlow's site I of ferric heme-HSA (ranging between 1.7×10^3 and $1.6 \times 10^5 M^{-1}$) are lower by one order of magnitude than those for drug binding to ferric heme-free HSA (ranging between 1.9×10^4 and $1.8 \times 10^6 M^{-1}$). According to linked functions, the value of the association equilibrium constant for heme binding to HSA decreases from $7.8 \times 10^7 M^{-1}$, in the absence of drugs to $7.0 \times 10^6 M^{-1}$, in the presence of drugs. These findings represent a clear-cut evidence for the allosteric inhibition of drug binding to HSA Sudlow's site I by the heme.

4.10.4 Protein composition

The plasma-derived products are subjected to various physio-chemical processes for increasing the safety profile of the product, as it either destroys the virus or removes it. The common method used is the pasteurization for viral inactivation. The intactness of the protein after passing through such rigorous processes has to be confirmed by zone electrophoresis.

4.10.5 Molecular size distribution : for determination of polymers and aggragtaes

A study was conducted on Anaphylactoid reactions to infusions of plasma protein and human serum albumin. Role of aggregated proteins and of stabilizers added during production by Ring J, Stephan W et al as published in Clin Allergy. 1979 Jan;9(1):89-97. Six patients suffering from anaphylactoid reactions after infusion of pasteurized plasma (PP) or human serum albumin (HSA) were investigated. Clinical symptoms ranged from urticaria and hypotension to cardiac arrest. Immunoglobulin levels, especially of IgA, were normal, as were concentrations of complement factors C3, C4 and factor B. In skin and lymphocyte transformation tests patients, with the

exception of one severely allergic to protein, did not react to the monomeric pure HSA. Five out of six patients reacted against HSA aggregates and three patients to the HSA modified by caprylate added as stabilizer during commercial HSA production. This study concluded that the anaphylactoid reactions developing after PP or HSA infusion result from a non-specific reaction to protein aggregates and in some cases possibly from a specific immune response to the caprylate-modified HSA.

4.10.6 Total Protein Concentration

Human albumin is produced at two concentrations. The 4–5% albumin solution is an isotonic solution particularly suitable for fluid replacement in hypovolaemia. The 20–25% albumin is a hypotonic but hyperoncotic solution for the treatment of fluid loss where electrolyte or fluid load is contraindicated.

5.0 Quality Control testing at National Institute of Biologicals- Blood Products Laboratory

India is a major importer of plasma derived medicines. A few Indian companies are importing bulk products and processing them indigenously and some are manufacturing such products using plasma available in the country. Since these are products derived from human plasma, consideration should be given to the following factors: i) Microbial contamination may occur and may lead to the accumulation of pyrogens; ii) Viruses and other adventitious agents may be introduced by reagents during manufacture (e.g., enzymes from tissue extracts or monoclonal antibodies used for affinity chromatography) iii) The methods of manufacture may introduce process related impurities such as proteins, solvents, detergents, and antibodies or other ligands from chromatography; iv) Methods of manufacture may modify the product resulting in adverse consequences for recipients, for example by the formation of product related impurities, such as neo-antigens, or by compromising the biological activity of the active component, e.g. by activation of coagulation factors leading to enhanced thrombogenicity. This is particularly of concern for steps introduced to inactivate or remove viral contamination which may affect the quality or yield of products

In view of the above facts, to ensure the safety and efficacy of such drugs derived from human plasma, National Institute of Biologicals has set up the Blood Products laboratory for quality control testing as per the Pharmacopoeal requirements and became functional in the year 2002. The major functions of the laboratory are: a) Quality control

testing of plasma derived products b) Examination of summary production protocol for issue of test reports and c) Joint inspection of manufacturing facilities with the team of Drugs Control Office. The laboratory aims to become a strong science based support system for the regulatory bodies of the country to develop and implement policies related to the regulation and quality management of these products.

5.1 Infrastructure:

The laboratory is equipped with instruments like High Performance Liquid Chromatography (HPLC), UV-Visible spectrophotometer, Micro plate Reader, Micro plate washer, Vertical Electrophoresis System, pH meter, Kjeldahl apparatus, Vacuum Oven, Osmometer, Digital Water bath with external temperature recorder, Incubator, Under Counter Freezer, Electronic Weighing Balance and other basic facilities like, Bio-safety cabinets, Fume hood etc. Major equipment are placed under annual maintenance contract (AMC) and calibrated annually by an NABL accredited laboratory or equipment specific vendors as per preventive maintenance schedule.

5.2 SAMPLE REQUIREMENT FOR QUALITY CONTROL TESTING AT NIB

Number of vials required for batch testing

S.No	Product	No. of vials required for a batch
1.	Human albumin 25 %, 20 % , 5 %	06 Bottles

5.3 DOCUMENTS TO BE SUBMITTED BY THE MANUFACTURER/ IMPORTER DURING SAMPLE SUBMISSION

	Requirement
1	Batch release from country of origin/ Batch release exemption from the country of origin
2	Plasma pool certificate from NCL/ manufacturer
3	Summary information on the plasma pool {Summary Protocol for Production and Testing]
	A. Source of plasma
	B. Period of collection of plasma
	C: Certificate of compliance to International standards for Collection, Processing, storage and Quality control of Human Plasma for Fractionation
4	Name of three countries
5	Method of viral inactivation
6	Certificate of analysis with name of pharmacopoeia complied

5.4 TESTS CONDUCTED AT NIB FOR QUALITY CONTROL TESTING OF HUMAN ALBUMIN:

SI No.	Test(s) conducted	Specifications
1	Identification	Precipitation band only with anti-human sera (IP 2010)
2	Purity by SDS-PAGE	No impurity (any band other than the main band) in the electropherogram obtained with the test solution may be more intense than the other impurity band obtained with the reference solution (qualitative analysis). [NIB/BPL/SOP/12/R3]
3	Total protein by Biuret	Not less than 95% protein and not more than 105% protein as stated on the label (IP 2010)
4	pH	6.7 – 7.3 (IP 2010)
5	Haem content	Not more than 0.15 at 403 nm (IP 2010)
6	Stability	Contents of the final container remain unchanged as determined by visual inspection after heating at 57°C for 50 hours (IP 2010)
7	Molecular size Distribution by HPLC	Area of peak due to polymer and aggregates is not greater than 10% of total area of the chromatogram (BP 2012)
8	Abnormal Toxicity	Complies test for abnormal toxicity (IP 2010)
9	Sterility	Complies with test for sterility [NIB/STR/SOP/01/R1]
10	HIV 1 & 2 antibody	Absence of HIV antibodies (IP 2010), Non-reactive [Drugs & cosmetic act & rules, Schedule F , Part XII-C (G)]
11	HCV antibody	Non-reactive [Drugs & cosmetic act & rules, Schedule F , Part XII-C (G)]
12	HBsAg	Non-reactive

		[Drugs & cosmetic act & rules, Schedule F , Part XII-C (G)]
13	Pyrogen	Complies with the test for pyrogen (IP 2010)
14	Sodium*	Not more than 160 mmole/L
15	Potassium*	Not more than 2 mmole/L
16	Protein composition *	In the electropherogram obtained with the test solution on <i>cellulose acetate</i> or on <i>agarose gel</i> , not more than 5% of the protein has a mobility different from that of the principal band [IP 2010]

*To be initiated since April 2013

5.4 Release of the Certificate of analysis

The reports of each test are compiled and verified with regard to the validity of the test and compliance to the specifications as per the Indian Pharmacopoeia/ British Pharmacopoeia & Drugs & cosmetic act & rules, Schedule F , Part XII-C (G). The Certificate of analysis is given below:



NATIONAL INSTITUTE OF BIOLOGICALS
(Ministry of Health & Family Welfare)
An ISO 17025: 2005 Accredited Institute



Certificate No: T- 2010



Certificate No: T- 2011

F.No.: N.3- /2013-SRRD/BP

Date

CERTIFICATE OF ANALYSIS

Date of sample receipt :
Start date of analysis :
CDR No. :
Analytical Report No :
Name of the product and dosage form :
Name of the manufacturer :
Batch / Lot No :
Manufacturing date :
Expiry date :

Sl No.	Test(s) conducted	Specifications	Results
1	Identification	Precipitation band only with anti-human sera (IP 2010)	
2	Purity by SDS-PAGE	No impurity (any band other than the main band) in the electropherogram obtained with the test solution may be more intense than the other impurity band obtained with the reference solution (qualitative analysis). [NIB/BPL/SOP/12/R3]	
3	Total protein by Biuret	Not less than 95% protein and not more than 105% protein as stated on the label (IP 2010)	
4	pH	6.7 – 7.3 (IP 2010)	
5	Haem content	Not more than 0.15 at 403 nm (IP 2010)	

6	Stability	Contents of the final container remain unchanged as determined by visual inspection after heating at 57°C for 50 hours (IP 2010)	
7	Molecular size Distribution by HPLC	Area of peak due to polymer and aggregates is not greater than 10% of total area of the chromatogram (BP 2012)	
8	*Abnormal Toxicity	Complies test for abnormal toxicity (IP 2010)	
9	Sterility	Complies with test for sterility [NIB/STR/SOP/01/R1]	
10	*HIV 1 & 2 antibody	Absence of HIV antibodies (IP 2010), Non-reactive [Drugs & cosmetic act & rules, Schedule F, Part XII-C (G)]	
11	*HCV antibody	Non-reactive [Drugs & cosmetic act & rules, Schedule F, Part XII-C (G)]	
12	*HBsAg	Non-reactive [Drugs & cosmetic act & rules, Schedule F, Part XII-C (G)]	
13	Pyrogen	Complies with the test for pyrogen (IP 2010)	

*THE TESTS MARKED ARE NOT ACCREDITED BY NABL

CONCLUSION Based on the findings of the tests mentioned above, the product _____, Batch / Lot No: _____, comply / ~~do not comply~~ the above requirements as mentioned in the respective SOPs / IP 2010 / BP 2012 / Drugs & cosmetic act & rules, Schedule F, Part XII-C (G).

Signature of the analyst

Signature of Lab head

5.5 ASSURING THE QUALITY OF TEST REPORTS

Laboratory quality control Program- Laboratory quality control (In-House control) is an essential aspect of ensuring that released is fit for the purpose determined by the quality objectives (ie. Accuracy and precision). When Properly executed the quality control samples can monitor various aspects of data quality on routine basis. In instances where performance falls outside acceptable limits, the data produced can be questioned and after investigation a determination made as to its validity. The dual foundations of the laboratory quality control program is its internal quality control, composed of day-to-day and sample-set to sample-set monitoring of analytical performance. The quality control data generated is recorded in such manner to detect trends.

Internal quality control samples are used to measure accuracy, precision, contamination and matrix effect. QCs are run per batch.

Quality Control Charts

Accuracy and precision control charts are used to determine if the measurement system process is in control and whether the results generated by the measurement system are acceptable. The control chart provides the tool for distinguishing the pattern of indeterminate (random) variation from the determinate (assignable cause) variation. This technique displays the test data from a process or method in a form which graphically compares the variability of all test results with the average or expected variability of small

groups of data, in effect, a graphical analysis of variance. The average or mean value is calculated and the spread (dispersion or range) is established. Common practice sets the warning limits at ± 2 standard deviations while control limits are set at ± 3 standard deviations on each side of the mean. Since the distribution of averages exhibits a normal form, the probability of results exceeding the control limits is readily calculated. The control chart is actually a graphical presentation of QC efficiency. If the procedure is *in-control*, the results will almost always be within established control limits. Further, the chart will disclose trends and cycles from assignable causes which can be corrected. It is emphasized that there is absolutely no substitute for sound judgment based on an appreciation of the analytical system, the technique, the quality control materials utilized, and the analytical interpretation of the data generated by the procedure.

Accuracy charts (other names are Mean Chart, Levy (Levey)-Jennings or Shewhart Control Chart) - The data from a series of analytical tests are plotted with the vertical scale in units such as percent (percent recovery), and the horizontal scale in units of batch number or time. The mean and standard deviation is calculated on the data. Upper and lower control limits are established at the mean $\pm 3X$ (where X is the calculated standard deviation). Upper and lower warning limits are established at the mean $\pm 2X$.

Precision charts (other names are Range Chart or R-chart) – The data from duplicates are plotted with the vertical scale in units such as percent (RPD), and the horizontal scale in units of batch number or time. The mean and standard deviation is calculated on the data. The upper control limit is established at the mean $X 3.27$ and the upper warning limit is established at the mean $X 2.51$. Precision control charts do not have a lower warning and control limit.

5.6 PROCEDURE FOR REPEAT TESTING AND INVESTIGATION OF OUT-OF – SPECIFICATION RESULTS

Criteria for repeat testing: The test is repeated when:

- The test is Invalid
- The test is valid but the results of the sample is out of specification

Invalid Test (Fig 4)

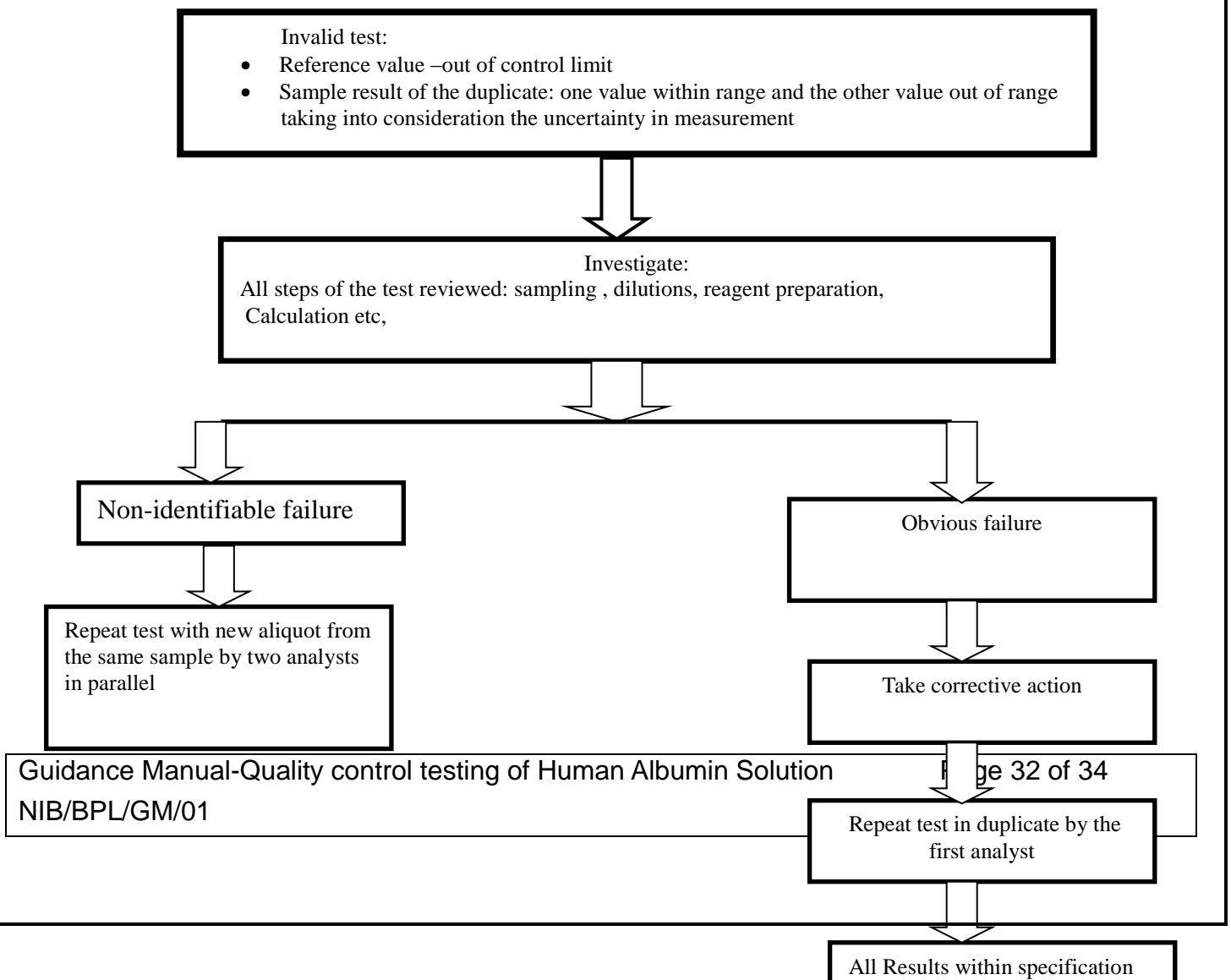
- The test is invalid if the measured values of the reference/ in-house control do not comply with the specifications and this may imply a systemic error.
- One of the results of the duplicate test sample showing out of range while the other is within specification taking into account the uncertainty in measurement of that particular test.
- The supervisor shall review and discuss in depth with the performer, the execution of the entire analytical testing procedure, equipment and calculation used.

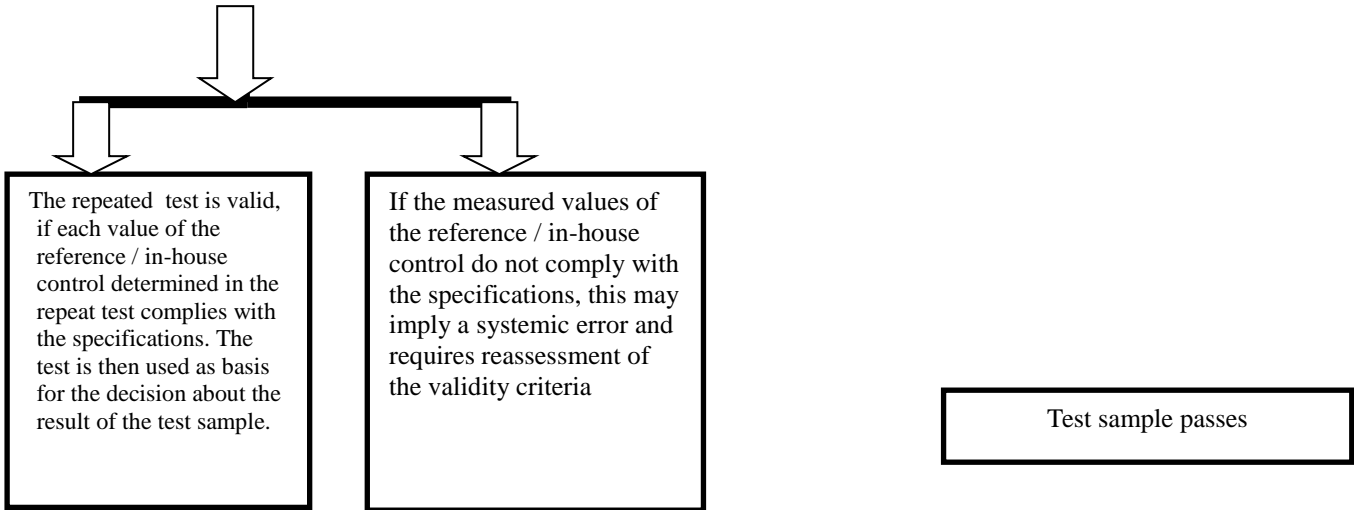
Valid test but sample gives out of specification result (Fig 5)

- If the test is valid and no obvious failure can be found, the Out-of -specification result is probably due to the test sample itself and hence to confirm this, the test has to be repeated.

PROCEDURE FOR REPEAT TESTING AND INVESTIGATION OF OUT-OF -SPECIFICATION RESULTS (Fig: 4)

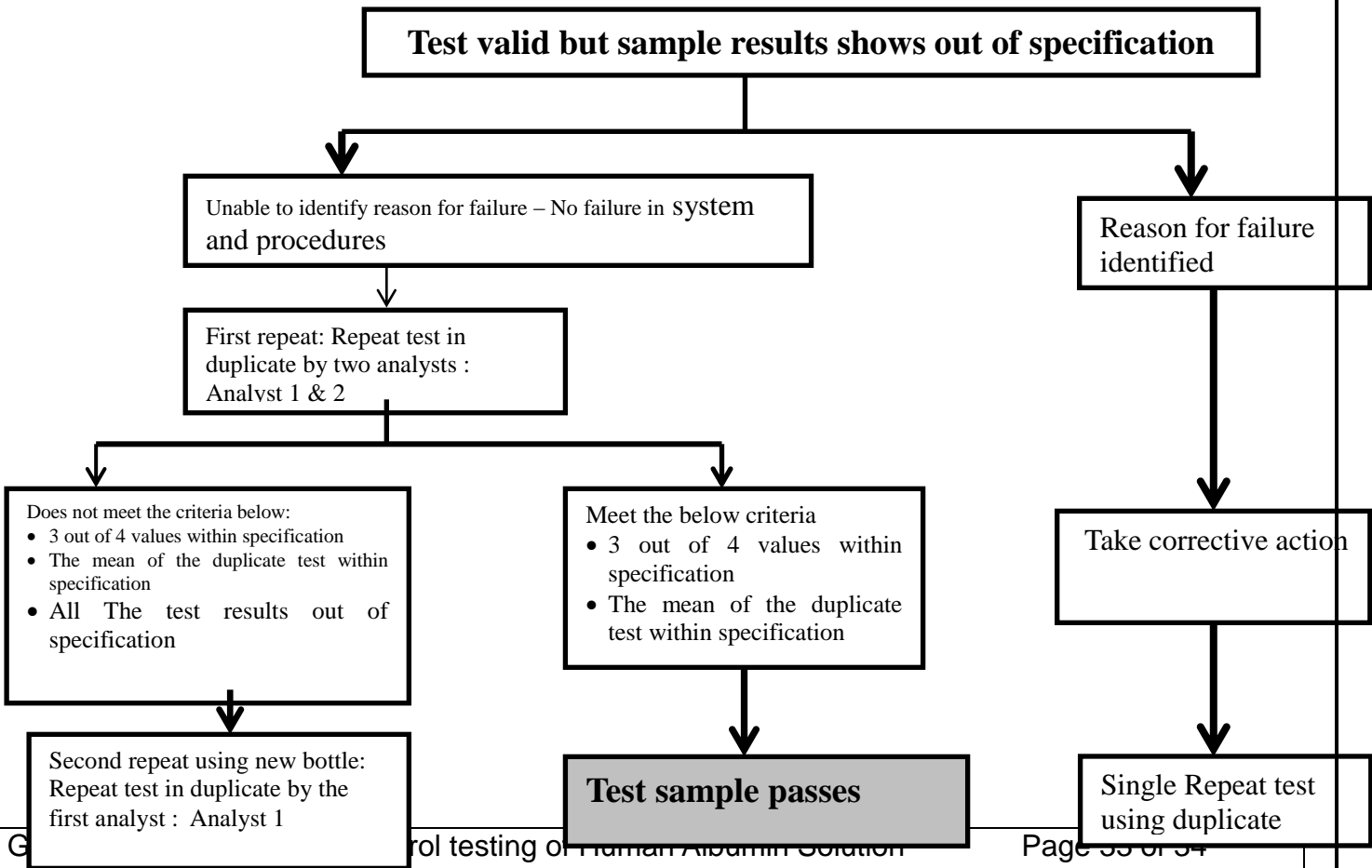
INVESTIGATION OF INVALID TEST

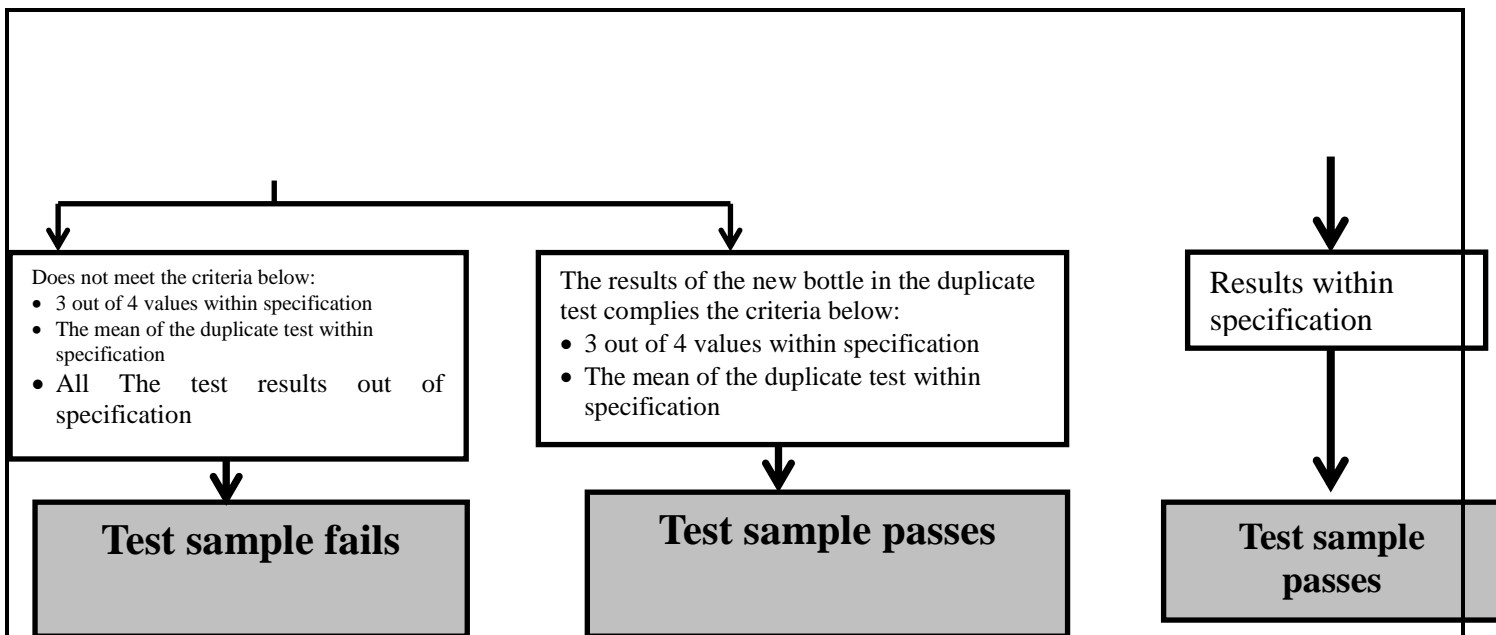




**PROCEDURE FOR REPEAT TESTING AND INVESTIGATION OF
OUT-OF –SPECIFICATION RESULTS (Fig : 5)**

OUT-OF –SPECIFICATION OF TEST SAMPLE RESULTS





6.0 References:

- i. Indian Pharmacopoeia 2010
- ii. Production of human albumin solution: a continually developing colloid: British Journal Anesthesia 2000; **85**: 887–95
- iii. Curling JM. Albumin purification by ion exchange chromatography. In: Curling JM, ed. Methods in Plasma Protein Fractionation. London: Academic Press, 1980; 77–92
- iv. Guideline on core SPC for Human Albumin Solution, CHMP, European Medicines Agency, 2005
- v. Note for guidance on plasma Derived medicinal products, CHMP, European Medicines Agency, 2001
- vi. The Role of Albumin in Fluid and Electrolyte Balance; Journal of Infusion Nursing September/October 2006 ; Volume 29 Number 5; Pages 260 - 265