DATA SHEET

ALHYDROGEL™ - Aluminium hydroxide gel adjuvant

Alhydrogel is available in three standard versions:

1. Alhydrogel 1.3% (1.3% ash residue dry matter, Al_2O_3 equivalent to a calculated $Al(OH)_3$ concentration of 2.0%).
2. Alhydrogel 2.0% * (2.0% ash residue dry matter, Al_2O_3, equivalent to a calculated $Al(OH)_3$ concentration of 3.0%).

Alhydrogel 1.3% and 2.0% differs only in their concentration and in the directly derived parameters, such as the protein adsorption capacity per ml.

Unless otherwise agreed in writing and confirmed when placing the order, the goods shall be delivered to the customer no later than 6 months prior to the sales expiry date stated on the label and the certificate of analysis.

ALHYDROGEL™ - Specifications of the different types

Test parameter	Acceptable range	
	ALHYDROGEL 1.3%	ALHYDROGEL 2%
Ash residue (Al_2O_3)	Min. 1.23% Max. 1.37% w/w	Min. 1.90% Max. 2.10% w/w
Corresponding to $Al(OH)_3$	Min. 1.88% Max. 2.10% w/w	Min. 2.91% Max. 3.21% w/w
Al	Min. 5.9 mg/ml Max. 7.1 mg/ml	Min 9.0 mg/ml Max. 11.0 mg/ml
N	Max. 0.005% w/w	Max. 0.005% w/w
SO_4 (free)	Max. 0.05% w/w	Max. 0.05% w/w
SO_4 (total)	Max. 0.1% w/w	Max. 0.1% w/w
Fe (total)	Max. 15 ppm	Max. 15 ppm
pH (at the time of production)	Min. 6.0 Max 7.0	Min. 6.0 Max 7.0
Sterility	No growth in test samples	No growth in test samples
Pyrogenicity in 3 rabbits	Max. 1.15 °C	Max. 1.15 °C
(Pyrogenicity in 6 rabbits	Max. 2.80 °C)	Max. 2.80 °C)
Protein adsorption capacity	16 ± 3 mg HSA/ml	23 ± 5 mg HSA/ml

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**) This formulation was chosen as international research standard by an independent scientific workshop in 1988. (D.E.S. Stewart-Tull (1989): Recommendations for the assessment of adjuvants (Immunopotentiators) In: Immunological Adjuvants and Vaccines, ed. G. Gregoriadis; A.C. Allison and G. Poste; Plenum Publishing Corp.)*

ALHYDROGEL 1.3 %

	Value found	Acceptable range
Ash residue (Al_2O_3)	1.35 %	Min. 1.23 % Max. 1.37 % w/w
Corresponding to $Al(OH)_3$	2.07 %	Min. 1.88 % Max. 2.10 % w/w
Al/ml (EDTA titration)	6.8 mg	
N	0.003 %	Max. 0.005 % w/w
SO_4 (free)	0.01 %	Max. 0.05 % w/w
SO_4 (total)	0.04 %	Max. 0.1 % w/w
Fe (total)	<5 ppm	Max. 15 ppm
pH (at the time of production)	6.4	Min. 6.0 Max 7.0
Sterility	OK	No growth in test samples
Pyrogenicity in 3 rabbits	0.8 °C	Max. 1.15 °C
Protein adsorption capacity	15 mg HSA/ml	15 ±3 mg HSA/ml

ALHYDROGEL 2 %

	Value found	Acceptable range
Ash residue (Al_2O_3)	1.98 %	Min. 1.90 % Max. 2.10 % w/w
Corresponding to $Al(OH)_3$	3.03 %	Min. 2.91 % Max. 3.21 % w/w
Al/ml (EDTA titration)	10.4 mg	
N	0.001 %	Max. 0.005 % w/w
SO_4 (free)	0.01 %	Max. 0.05 % w/w
SO_4 (total)	0.07 %	Max. 0.1 % w/w
Fe (total)	<5 ppm	Max. 15 ppm
pH (at the time of production)	6.3	Min. 6.0 Max 7.0
Sterility	OK	No growth in test samples
Pyrogenicity in 3 rabbits	0.7 °C	Max. 1.15 °C
Protein adsorption capacity	20 mg HSA/ml	21 ±5 mg HSA/ml

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ALHYDROGEL

Aluminium Hydroxide Gel

Product #	Description	Host/Presentation Packing	
A-1090S	Alhydrogel™, Grade A, Sterile		250 ml.
A-1090S	Alhydrogel™, Grade A, Sterile	6 vials or more	250 ml.
A-1090BS	Alhydrogel™, Grade B, Sterile		250 ml.
A-1090BS	Alhydrogel™, Grade B, Sterile	6 vials or more	250 ml.

Description

Alhydrogel is an aluminium hydroxide gel. It is a stable, viscous homogenous material retaining its properties even after storage for several years.

Characteristics

Alhydrogel is available as a sterile pyrogen free product with a high degree of purity and meets all the specifications of the British Pharmacopoeia except in its neutralising capacity. It should therefore not be used as an antacid.

The special techniques used in its manufacture ensure a stable gel which has a uniformly high adsorption capacity and which can be repeatedly autoclaved without losing any of this adsorption capacity. As the product itself is a poor heat conductor care is necessary during autoclaving and constant stirring is advised.

Alhydrogel should always be stored in containers of aluminium, pyrex glass or inert plastic as the adsorption capacity of Alhydrogel can be affected by impurities present in the material of other types of containers.

At concentrations of 6 mg of aluminium hydroxide per ml Alhydrogel exhibits a slower sedimentation rate than other similar products commercially available. The gel stability and re-suspension characteristics of vaccines containing Alhydrogel remain satisfactory for long periods, even for 3 or 4 years whether maintained under refrigerated conditions or at room temperature. However freezing

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can completely destroy the colloidal nature of the gel, and hence must be avoided.

Alhydrogel normally carries a positive charge in the absence of electrolytes, however, it readily forms complexes with aminoacids the overall charge of which depends upon the particular aminoacid. The specific adsorptive properties of a complex differs from that of the untreated Alhydrogel in a manner dependent upon the characteristics of the particular aminoacid.

The adsorptive capacity of Alhydrogel is influenced by the type and concentration of electrolytes present. Monovalent anions such as chlorides, acetates and lactates have little effect especially at low concentrations. Multivalent anions, however, such as phosphates, sulphates and borates appreciably reduce the adsorptive capacity, particularly at neutral or alkaline pH values.

The great ease with which some adsorbed materials can be eluted from Alhydrogel makes it an extremely useful tool in any biochemical laboratory. Differential removal of adsorbed material can be accomplished by gradient elution. An ideal electrolyte for this purpose is ammonium sulphate.

Adjuvant for Immunization

Having been in use for many years Alhydrogel is now well established as an adjuvant for use in vaccine production. During this time no adverse reactions have been observed which can be attributed to the Alhydrogel.

Factors Influencing Adsorption onto Alhydrogel

It is recommended that experimental assays are carried out to determine the optimum conditions for the adsorption of a given antigen onto Alhydrogel.

In general, Alhydrogel has a positive charge under biological conditions and therefore it will readily adsorb most substances which are negatively charged e.g. most proteins at neutral pH. A number of factors, however, greatly influence the degree of adsorption. The most important of these are:

(1) Concentration and nature of antigen and accompanying substances.
(2) pH of the resulting mixture.
(3) Concentration of salts and buffering ions.

(1) Batch to batch variations in the antigen concentration should be minimized if a standard formula i.e. standard content of Alhydrogel is to be followed. Of equal importance when working with semipurified antigens is the concentration of accompanying substances like proteins. Such proteins may compete with the

antigen in the adsorption process. In many cases, a useful parameter for the standardization of adsorption is any assay of the total amount of protein (e.g. by the biuret method) in the solution to be adsorbed.

(2) Antigens to be adsorbed are usually amphoteric substances. Thus it is evident that the pH in the final product is a determining factor for the net charge of the antigen and consequently for the binding affinity to Alhydrogel. Furthermore the net charge of Alhydrogel is also influenced by pH. The adsorptive capacity of Alhydrogel is therefore determined by factors resembling the situation well known in ion-exchange chromatography. For optimal results, the binding of a given antigen should be investigated at different pH-values, and preferably with 0.5 pH intervals within the acceptable physiological range.

(3) As in the case of ion-exchange chromatography the concentration of salts and buffering ions plays a major role i.e. high concentrations tend to lower the adsorptive capacity of Alhydrogel. At isotonocity, however, only minor effects are usually seen. Care must be taken to avoid high concentrations of phosphate and similar ions i.e. sulphates and borates. For instance, phosphate has a marked influence on adsorption. In fact many antigens can be quantitatively eluted from Alhydrogel by phosphate concentrations exceeding 0.3 M.

Assay of Adsorptive Capacity

Alhydrogel is tested for adsorptive capacity according to the procedure described on page 7. This assures a standardized product of reproducible quality.

It is suggested that a similar procedure should be followed when assaying the adsorptive capacity of Alhydrogel for an antigen for vaccine production. In most cases precipitating antisera against the antigen to be adsorbed are available and hence immunoelectrophoresis represents an easy, quick and reliable method for the quantitation of antigen adsorption.

General Information

When delivered, Alhydrogel has an extremely low ionic strength, and in many cases it may be advisable to adjust the pH by adding an adequate buffer like glycine, **before** mixing the antigen with the Alhydrogel. This is of special interest when working with antigen which is sensitive to a low pH (e.g. foot-and-mouth disease virus), as the Alhydrogel at the delivery has a pH of the order of pH 6.

It may be necessary to re-sterilize Alhydrogel prior to use and in view of its very poor heat conductivity, it is strongly recommended that this sterilization should take place in a stirred vessel and be effected by heating at 121° Centigrade for one hour.

Alhydrogel is supplied in the following sizes of packing:

Aluminium Bottles of 250 grammes net
Polyethylene Containers (inert plastic) of 5 kg net
Polyethylene Containers (inert plastic) of 25 kg net

Each container is packed in a cardboard box for extra protection.

The 25 kg polyethylene containers are also available in unit-loads packed on pallets and shrinkwrapped. One pallet contains 24 containers and the unit-load is consequently 600 kg net. The gross weight including pallet is 680 kg.

The Chemical Analysis is as follows:

		Grade »A«	Grade »B«
Dry Matter as Al_2O_3		1.3 %	2.0 %
Equivalent to Al $(OH)_3$		2.0 %	3.0 %
Conductivity	max.	0.50 mS	0.50 mS
Nitrate as N	max.	0.005 %	0.005 %
Free SO_4	max.	0.05 %	0.05 %
Total SO_4	max.	0.1 %	0.1 %
pH of the order of		6-7	6-7

The Adsorption Capacity is tested by means of Rocket Immunoelectrophoresis (see page 7).

Otherwise, Alhydrogel meets all the specifications of the British Pharmacopoia - except in its neutralising capacity.

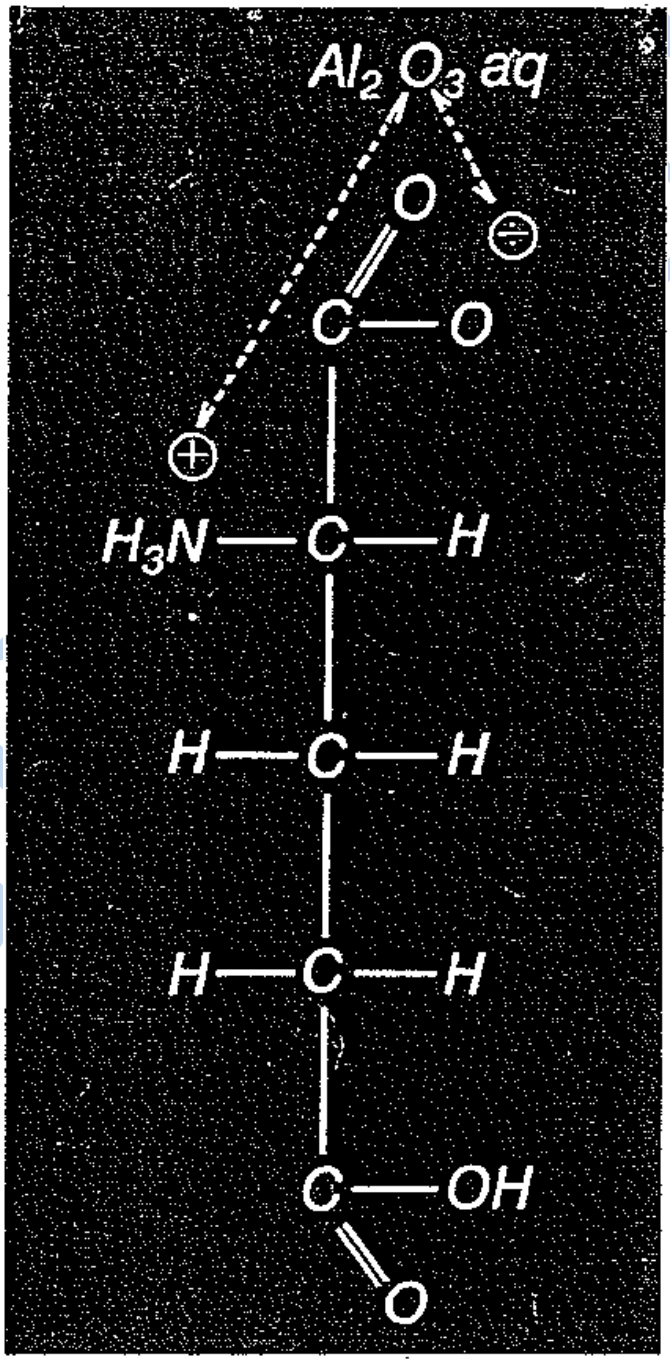
The Formation of Complexes with Alhydrogel

Alhydrogel/Amino Acid Complexes

Amino acids are readily adsorbed onto Alhydrogel to form complexes. These complexes are themselves adsorbant and their specificity is determined by the amino acid.

Alhydrogel / Glutamic Acid Complex

While Alhydrogel is itself not selective towards serum proteins, when complexed with glutamic acid its characteristics are markedly different. The complex ALHYDROGEL - GLUTAMIC ACID has a great affinity towards ß-lipoproteins and this complex is then of value in the purification of gamma globulin. Under suitably controlled conditions pyrogenic material will also be adsorbed at the same time as the ß-lipoprotein.



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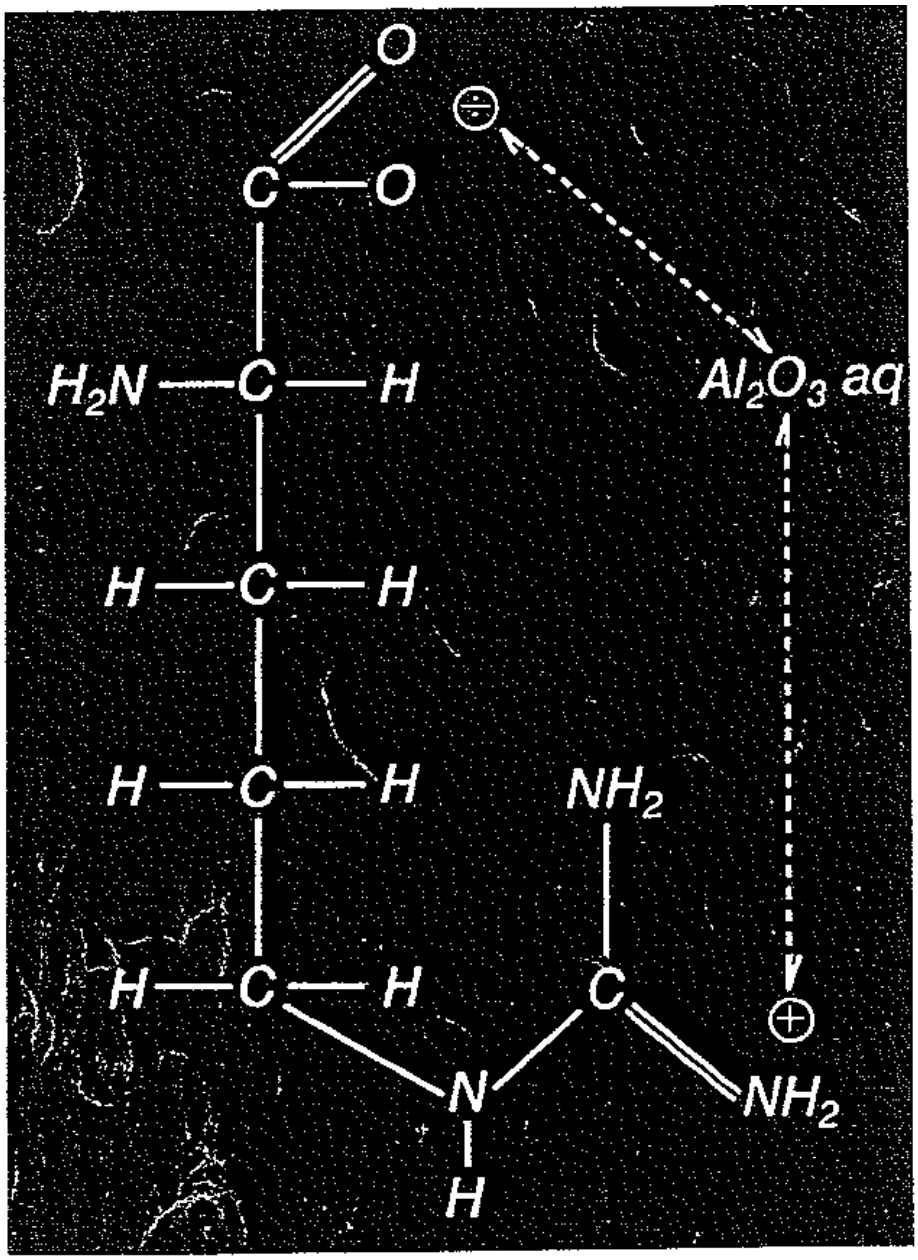
Alhydrogel / Arginine Complex

The amino acid arginine is strongly basic and adsorbes onto Alhydrogel. The complex, which is characterized by the guanidine groups of arginine is similarly basic.



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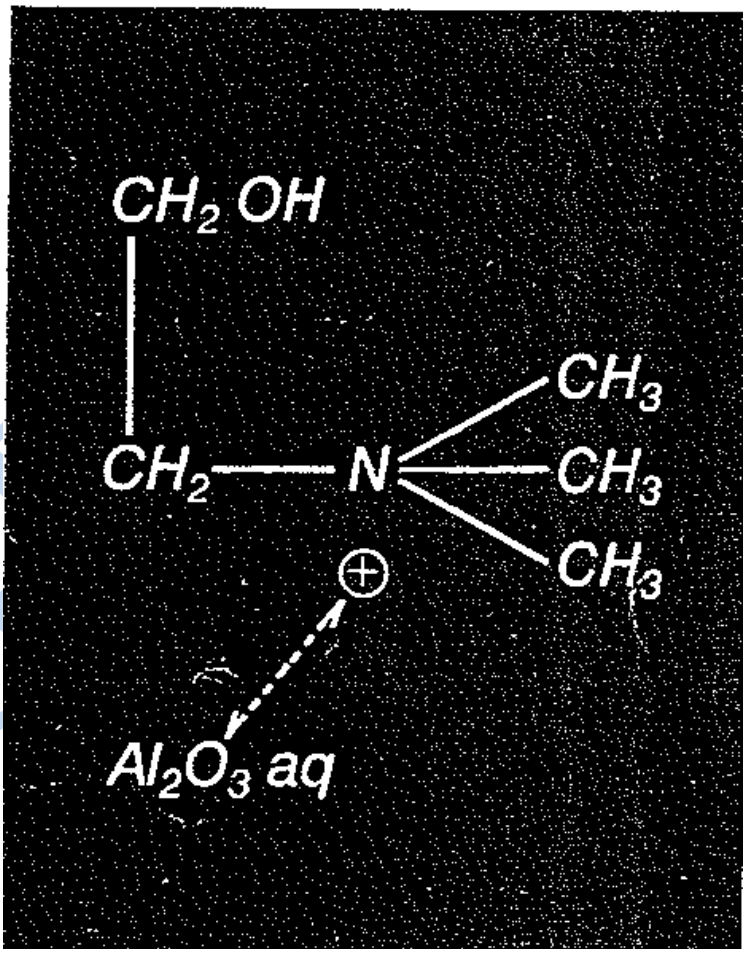
Alhydrogel/Methionine Complex

The amino acid methionine is characterized by its sulphur atom and this is significant in the ALHYDROGEL - METHIONINE COMPLEX. Here a selective adsorption for the copper containing protein »ceruloplasmin«, is developed, and it is possible to prepare pure ceruloplasmin by these adsorption techniques.

Alhydrogel/Cholin Complex

A further example of an Alhydrogel Complex can be illustrated by that formed with cholin. Here a strongly basic adsorbant is formed with characteristic properties. This complex is selective for negatively charged proteins, and has proved of value in the fractionation of organo-hydrolysates e.g. in thymus and pancreas.



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Alhydrogel/Phosphate Complex

Alhydrogel itself carries a positive charge, but it may be altered to a negatively charged material by acidification with condensed phosphoric acid. By this procedure material with adsorptive properties which are the complete reverse of the original is obtained. It will in fact flocculate when mixed with the original Alhydrogel.

Test for Adsorption of Alhydrogel

The adsorption capacity of each batch of Alhydrogel is tested by means of a model protein by incubation with various concentrations of the Alhydrogel to be tested.

The contents of antigen in the aqueous phase is tested by means of rocket immunoelectrophoresis in agarose gels containing an antiserum against the model antigen.

If a precipitating antiserum against the antigen component in a given vaccine formulation is available, a similar test can be carried out using the specific antigen in question.

If details of methods are required, we shall be pleased to supply all necessary specifications.

Literature:

1. Weeke, B. Rocket Immunoelectrophoresis. Scand. J.Immunol. 2, suppl.1, 37-46,1973.
2. Weeke, B. Weeke, E. & Löwenstein, H. The adsorption of serum protein to aluminium hydroxide gel examined by means of Quantitative Immunoelectrophoresis. Scand. J. Immunol. 4, Suppl. 2, 149-154, 1975.

Experimental Procedure to Determine Optimal Conditions for Adsorption

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Mixtures of proteins or antigens can be separated by flocculation with Alhydrogel when the optimum concentration has been determined. One of the best and easiest methods to determine flocculation is as follows:

Portions of 10 mls. of the protein solution are transferred by pipette to a series of 12 test tubes, to which distilled water is then added in amounts from 11 to 0 mls. Finally a dilution of Alhydrogel (for instance 1 + 9) in distilled water is added in amounts increasing from 1-12 ml. The total amount in each tube will then be 22 ml. After cautious but rapid mixing and standing for some minutes one of the tubes will show a marked flocculation, the floccules sedimenting and leaving clear supernatent. Should the protein content of the solution not be ideal for the adsorption under the conditions chosen the flocculation will appear in the first or the last tube, and in such a case it is necessary to make a new test with a more suitable dilution of the protein or Alhydrogel.

(* proper dilution of alhydrogel and protein

STORE AT ROOM TEMPERATURE

AVAILABILITY

Alhydrogel is supplied in aluminium bottles as a homogenous viscous gel containing 2% Al $(OH)_3$ equivalent to 1.3% Al_2O_3.

The product is sterile and pyrogen free and has the following composition:

Dry Matter as Al_2O_3	1.3%
N	0.004%
Free SO_4	0.02%
Total SO_4	0.08%
As	<1 ppm
Pb	<10 ppm
pH	of the order 7.0

References

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Alhydrogel (suggested reading):

Lindblad EB. (1995)
Aluminium Adjuvants. *In*: The Theory and Practical Application of Adjuvants: 21 - 35.
Ed. Stewart-Tull DES. John Wiley & Sons Ltd.

Gupta RK, Relyveld EH, Lindblad EB, Bizzini B, Ben-Efraim S, Gupta CK (1993).
In: Adjuvants - a balance between toxicity and adjuvanticity.
Vaccine vol. 11, 3: 293 - 306.

Weeke B, Weeke W, Løwenstein H. (1975)
The Adsorption of Serum Proteins to Aluminium Hydroxide Gel Examined by Means of Quantitive Immunoelectrophoresis.
In: Quantitative Immunoelectrophoresis: New Developments and Applications (ed NH Axelsen)
Universitetsforlaget, Denmark: 149 - 154.

Burrell LS, Lindblad EB, White JL, Hem SL (1999).
Stability of Aluminium-Containing Adjuvants to Autoclaving.
Vaccine 17: 2599 - 2603.

Rinella JV Jr, Workman RF, Hermodson MA, White JL, Hem SL (1998).
Elutability of Proteins from Aluminium-Containing Vaccine Adjuvants by Treatment with Surfactants.
J. Colloid and Interface Sci. 197: 48-56.

Seeber SJ, White JL, Hem SL (1991).
Predicting the adsorption of proteins by aluminium-containing adjuvants.
Vaccine vol. 9: 201-203.

Thiele GM, Rogers J, Collins M, Yasuda N, Smith D, McDonald TL. (1990)
An Enzyme-Linked Immunosorbent Assay for the Detection of Antitetanus Toxoid Antibody Using Aluminium-Absorbed Coating Antigen.
J. Clin. Labor. Anal., 4: 126-129.

ACCU-SPECS SHEET

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The great ease with which some adsorbed materials can be eluted from Alhydrogel makes it an extremely useful tool in any biochemical laboratory. Differential removal of adsorbed material can be accomplished by gradient elution. An ideal electrolyte for this purpose is ammonium sulphate.

Applications of Alhydrogel

AN ADJUVANT FOR IMMUNIZATION

Vaccines prepared by antigens adsorbed onto Alhydrogel have several advantages. The antigenic content is easier to standardise and the vaccine has good stability. In vaccine production Alhydrogel adsorbed antigens are therefore simple and more convenient to use. Moreover the degree of local reaction following immunization is reduced.

The adjuvant effect of Alhydrogel is being studied by produc ing antisera specific for many proteins. In this way, by st' ying the most suitable methods to produce these antisera, timal conditions under which Alhydrogel can be used as a juvant will be elaborated in future publications.

ALHYDROGEL / AMINO ACID COMPLEXES

Amino acids are readily adsorbed onto Alhydrogel t plexes. These complexes are themselves adsorb specificity is determined by the amino acid.

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