

CrCl₃ Coupling of HGG, KLH and POL to SRBC (Conjugate with CrCl₃ on Day of Use)

Materials

- CrCl₃ stocks a. 10% (w/v) in normal saline
 b. .1M in normal saline

1X PBS
 Normal Saline n.s.
 Sheep Red Blood Cells washed 4X in saline

*2% PVP-BSA
 2.12%
 2.12%*

Protocol for HGG and KLH:

1. Add together .1 ml packed SRBC, 1 ml antigen at "W" mg/ml in "X", and 1 ml of CrCl₃ at "Y" % in "Z". (see chart below)
2. Incubate with occasional mixing at r.t. ~ 25°C for 15 to 20 min.
3. Centrifuge and wash 3X with PBS.
4. Resuspend to desired concentration.

Protocol for POL:

1. Add 2 ml of 10% SRBC suspension in saline (.2 ml packed SRBC in 1.8 ml saline) and 100 λ of POL at 1 mg/ml., and 100 λ of CrCl₃ at .005M (5 λ of stock a CrCl₃ + 95 λ saline).
2. Incubate mixture at room temp for 15-20 min.
3. Centrifuge & wash 3X with PBS
4. Resuspend to desired concentrations.

	W	X	Y	Z	Comments
KLH	3	1 x PBS	0.05% (from stock b)	1 x PBS	Gave same H.A. titers as gluteraldehyde coated cells (very poor for plaquing) <i>Excellent hemagglutination titers as well.</i>
HGG	10	1 x PBS	0.025% (from stock b)	N.S.	Gave excellent H.A. and ^{Hem} NEM titers-excellent for plaquing
HGG	20	1 x PBS	0.001% (from stock b)	N.S.	Gave excellent H.A. and ^{Hem} NEM titers-excellent for plaquing. N.B. These conditions gave better direct plaques than above but not as clear developed
POL	1	N.S.	0.005M (from stock b)	N.S.	Excellent for hemagglutination & hemolysis but no good for plaquing.

Lister

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PERTUSSIS VACCINE ADSORBED E.P. (Per/Vac/Ads)

FORMULATION A suspension of killed Bordetella pertussis adsorbed to an aluminium hydroxide adjuvant and containing thiomersal as a preservative. The vaccine complies with the European Pharmacopoeia requirements for content of pertussis organisms and for potency.

PERTUSSIS VACCINE E.P. (Per/Vac)

FORMULATION As Pertussis Vaccine Adsorbed (see above) but without aluminium hydroxide.

INDICATIONS These vaccines are used for the active immunization against pertussis of young children for whom vaccination against diphtheria and tetanus is contra-indicated or not required.

DOSAGE The course of 3 doses should be started within the first year of life, but preferably not before the 6th month; the interval between the first and second dose should be 6 to 8 weeks and between the second and third, 4 to 6 months. Reinforcement of immunity to pertussis is not normally necessary.

METHOD OF INJECTION Shake container well. The dose of both vaccines is 0.5 ml by intramuscular or deep subcutaneous injection.

REACTIONS Local reactions such as transient erythema and tenderness are more frequent after injection of adsorbed vaccine than they are after plain pertussis vaccine. The adsorbed vaccine is also more liable to give rise to a small nodule at the injection site, which gradually disappears.

General reactions such as restlessness and irritability during the 24 hours following the injection may also occur, but are less common after the adsorbed vaccine. Administration of pertussis vaccines is very occasionally followed by screaming fits; encephalopathy is a well-known but rare complication. Adrenaline Injection 1/1000 should be available in case of an immediate allergic reaction.

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