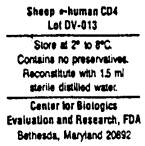

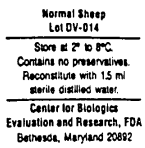

SPECIFICATIONS for FDA SHEEP POLYCLONAL SERA

Product Description

Each vial contains lyophilized sheep polyclonal serum specific for a particular antigen of Human Immunodeficiency Virus, its parent and degradation products. The materials in the vials are free of bacterial contamination and contain NO preservatives. When reconstituted in 1.5 ml sterile distilled water, the sera are neat with respect to unprocessed sera.

Methods of Production

Anti-Gag (p17 or p24) Sera: Antigens were purified using SDS-PAGE to resolve the proteins of HIV(3B). The pertinent band was excised, ground and emulsified in Freund's Complete Adjuvant for primary inoculation and in Incomplete Adjuvant for a booster administered 2 months later. Two additional booster doses in RIBI were given at the 3rd and 4th months followed by a final boost in Freund's Incomplete at 15 months. The animals were maintained by the Ungulate Unit at the NIH Animal Facility and plasmapheresed during the 16th and 17th months.

Anti-Env (gp120) and anti-CD4 Sera: Recombinant antigen produced and purified by Genentech was emulsified in Freund's Complete Adjuvant for primary inoculation and in Incomplete Adjuvant for the booster doses given at 1 and 4 months. The animal was plasmapheresed during the fifth month by the Ungulate Unit.

Plasmas were clotted using calcium chloride (10-20 mM final), strained to yield the sera and passed through a series of filters culminating at 0.22 microns. The sera were dispensed, lyophilized and tested for sterility by the Division of Product Quality Control, FDA.

Characterization of Sera

The final products were resuspended per label instructions and tested for sterility, potency and specificity as detailed on the obverse of this insert. The results are tabulated below.

Analysis	Test Sera with Lot Number in Parentheses				
	anti-p17 (DV-010)	anti-p24 (DV-011)	anti-gp120 (DV-012)	anti-CD4 (DV-013)	normal sheep (DV-014)
Sterility	No Growth	No Growth	No Growth	No Growth	No Growth
Potency reported as					
ELISA endpoint titer	1700	600	25000	10,000	-----
Neutralization titer	<8	<8	256	------	-----
Specificity assessed by					
Western Blot reaction (endpoint titer) to					
HIV-1:	p17/p55 ($10^{-4}/10^{-4}$)	p24/p55 ($10^{-3}/10^{-3}$)	gp120/160 ($10^{-4}/10^{-5}$)	------	-----
HIV-2:	No Reaction	p26 (10^{-1})	No Reaction	------	-----
Immunofluorescence endpoint titer with					
HIV_{3B}	320	80	640		
HIV^{RF}	80	20	640		
SIV_m	160	10	160		

Characterization Methods

Sterility was assessed as described in the Code of Federal Regulations, Title 21, 610.12.

Potencies of Lots DV-010, DV-011 and DV-012 were assessed by Enzyme-Linked Immunosorbent Assays (ELISA) using commercial HIV-1 antigen-coated plates. Sheep sera were diluted in 5% non-fat dry milk in PBS (Blotto) and dispensed in duplicate along with a dilution series of normal sheep serum and incubated at 37C for 1 hr. After 5x5 min washes with PBS containing 0.05% Tween 20, the plates were probed with a mixture of biotinylated anti-sheep IgG and avidin-derivitized horseradish peroxidase, each diluted 1:1000 in Blotto, again for 1 hr at 37C. After 6x5 min washes with PBS/Tween, the plates were developed for 30 min using o-phenylenediamine, fixed with 2N H_2SO_4 and read using a colorimetric plate reader. The specific antibody endpoint was assigned as that dilution yielding an optical density twice that of the comparable normal sheep serum dilution. The potency of Lot DV-013 was assessed using "Microfluor" plates (Dynatech, Inc.) coated with 20 ng/well of recombinant CD4 in PBS, alkaline phosphatase-labelled probes and methyl-umbelliferyl-phosphate substrate.

Neutralizing antibodies were measured by the microassay method of Vujcic et al (JID 157(5):1047) using HTLV-III_B infected H-9 cells.

Specificity was assessed by Immunoblot reaction using commercial HIV-1 antigen strips and the same reagents as described above, in the first instance, for ELISA except that the developing substrate was 4-chloro-1-naphthol. Reaction patterns produced by the sheep sera were compared for specificity with control patterns produced by monoclonal antibodies and endpoints were evaluated visually.

Specificity was also assessed by fixed-cell indirect immunofluorescence using HIV or SIV-infected H-9 cells. Test sera and controls were diluted in PBS, dispensed in 10 microliter droplets onto infected and uninfected cells fixed to microscope slides and incubated for 30 min at 37C. After 3x10 min rinses with PBS, 10 microliter droplets of FITC-labelled rabbit anti-sheep IgG diluted 1:40 in PBS/0.01% Amido Black were applied to all cell zones for a final incubation as above. The cells were scored for fluorescence, after final rinsing, and the endpoint assigned as the highest dilution of test serum yielding specific detectable fluorescence with infected, but not uninfected, cells.

Precautions

At low dilutions, all of the anti-HIV-1 sera react with degradation products or precursors of the specified HIV protein used to produce the antisera.