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Review Article **Volume 7 Issue 1**

QUANTIFICATION, CHARACTERIZATION AND IDENTIFICATION OF PROTEINS IN DIFFERENT VACCINES SAMPLE

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Abstract:

Aims and Objective

Estimation of protein concentration in Vaccine by Lowry's method.

Estimation of polysaccharide concentration in Vaccine by Orcinol method.

Determination of purity of Vaccine by SDS-PAGE.

* Coomassie Staining.

* Silver Staining.

Identification of Vaccine components by Western blot method

CONCLUSION

- Quantification of protein is done by Lowry's Assay.

  Correlation factor is 0.997.
- Characterization of polysaccharide is done by Orcinol Assay.

  Correlation factor is 0.998.
- SDS-PAGE
- In Coomassie staining band shows in between 35-45kD marker.
- Silver Staining is more sensitive which also shows its band in between 35-45kD marker.
- In Western Blotting the markers are more specific which confirms that the molecular weight of sample is 40kD.

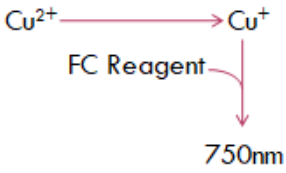
INTRODUCTION:

What is Vaccine?

- A vaccine is a biological preparation that improves immunity to a particular disease.
- It typically contains an agent that resembles a disease-causing microorganism.
- Usually made by whole organism or a part of an organism.
- ✓ Killed Vaccines ,Attenuated Vaccines, Conjugate Vaccine, Protein Vaccines.
- Conjugate Vaccine: Polysaccharide, Protein .
- Protein Vaccine

Estimaton Of Protein Concentration In Given Vaccine Samples By Lowry Method:

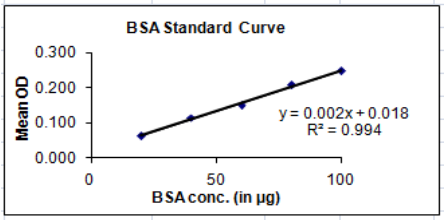
- Aromatic proteins reacts with copper sulphate under basic condition



Result :

Estimate the protein concentration by Lowry's method.

BSA Conc. (in µg)	OD at 750 nm		Mean OD
20	0.064	0.061	0.063
40	0.108	0.123	0.116
60	0.149	0.153	0.151
80	0.209	0.211	0.210
100	0.249	0.245	0.247

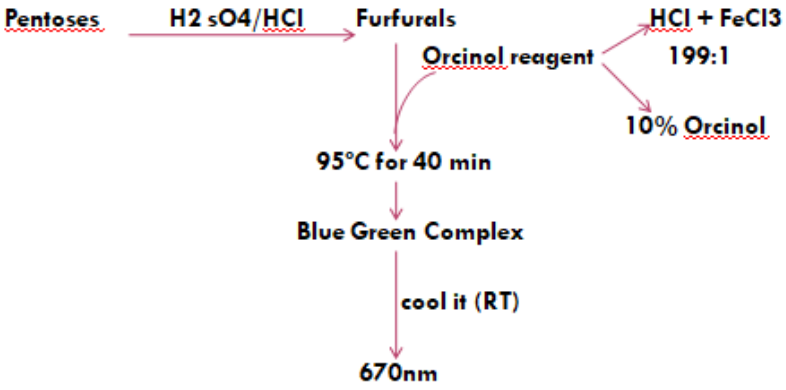


Intercept	0.018
Slope	0.002
Correlation	0.997

Samples	Volume taken (in	OD at 750 nm		Mean OD	Dil. Factor	Protein Conc.	Protein Conc.	Mean Protein Conc. (in mg/mL)	Standard Deviation	% RSD
Sample-1	50	0.082	0.088	0.085	20	576.91	0.577	0.58	0.011	1.90
	100	0.145	0.166	0.156	10	592.66	0.593			
Sample-2	50	0.089	0.085	0.087	20	594.17	0.594	0.58	0.022	3.88
	100	0.153	0.144	0.149	10	562.46	0.562			
Sample-3	50	0.1	0.072	0.086	20	585.54	0.586	0.56	0.039	7.03
	100	0.126	0.156	0.141	10	530.10	0.530			

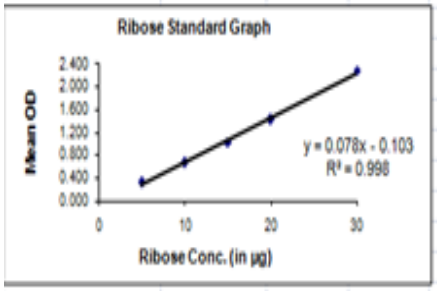
- PRP – Poly Ribosyl Ribitol Phosphate

- Photometric.
- Estimation of Carbohydrates.
- Pentoses.



Estimatation of ribose and PRP in solution containing polysaccharide/ proteins by Orcinol Method

Ribose Conc. (in µg)	OD at 670 nm		Mean OD
5	0.32	0.332	0.326
10	0.664	0.692	0.678
15	1.039	1.036	1.038
20	1.423	1.446	1.435
30	2.278	2.291	2.285

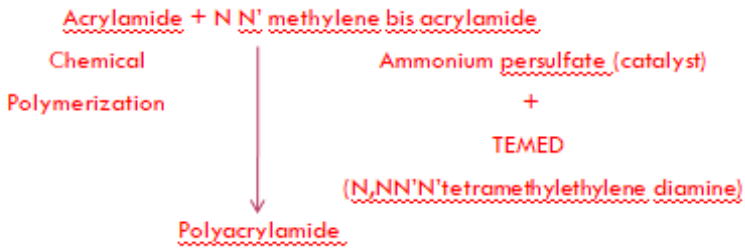


Slope	0.078
Intercept	-0.103
Correlation	0.998

Samples	Volume taken (in µL)	OD at 670 nm		Mean OD	Dil Factor	Ribose Conc. (in µg/mL)	PRP Conc. (in µg/mL)	Average PRP Conc. in µg/mL	Average PRP Conc. in mg/mL	Standard Deviation	% RSD
Sample-1	100	0.462	0.401	0.432	10	68.15	166.98	158.53	0.16	11.95	7.54
	200	0.864	0.852	0.858	5	61.26	150.08				
Sample-2	100	0.388	0.395	0.392	10	63.06	154.49	146.98	0.15	10.62	7.23
	200	0.778	0.802	0.790	5	56.92	139.46				
Sample-3	100	0.404	0.385	0.395	10	63.44	155.43	145.73	0.15	13.72	9.41
	200	0.751	0.785	0.768	5	55.52	136.03				

SDS-PAGE TO DETERMINE PURITY OF VACCINE

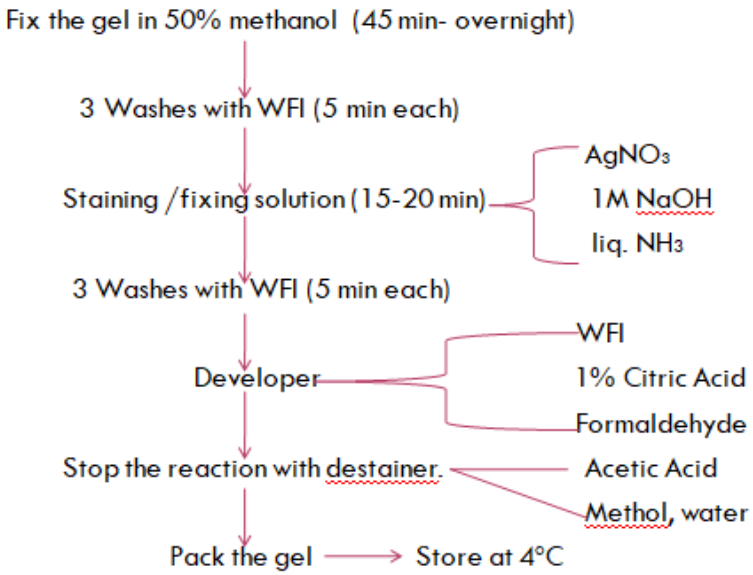
SDS-PAGE - Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis



- Separate proteins according to their electrophoretic mobility.
- Analyzed is first mixed with SDS.
- ✓ Anionic detergent.
- ✓ Denatures secondary and non–disulfide–linked tertiary structures.
- ✓ Negative charge to each protein in proportion to its mass.
- The SDS binds to the protein in a ratio of approximately 1.4 g SDS per 1.0 g protein.
- ✓ uniform mass:charge ratio for most proteins
- RESOLVING GEL Tris, Glycine, Acrylamide, Bis acrylamide,
- STACKING GEL SDS, APS, TEMED, Glycerol.
- Tracking dye also added.

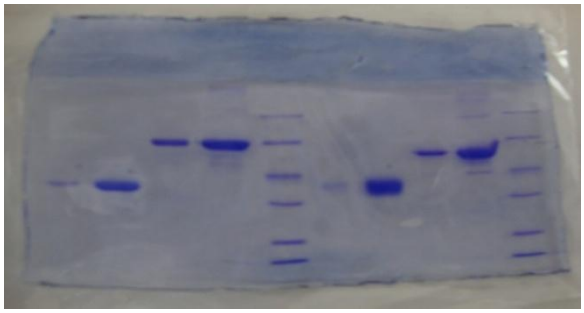
STAININ

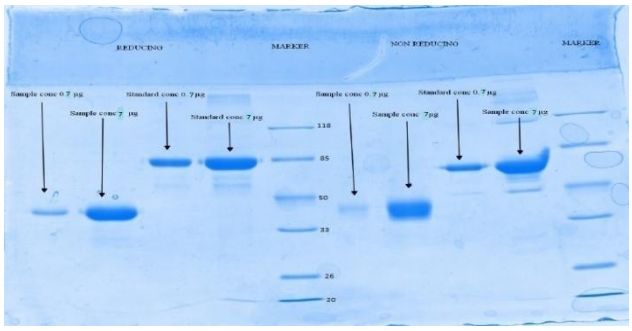
- Coomassie Staining.
- Silver Staining.
- ➢ Depends on reduction of ionic silver to its metallic form.



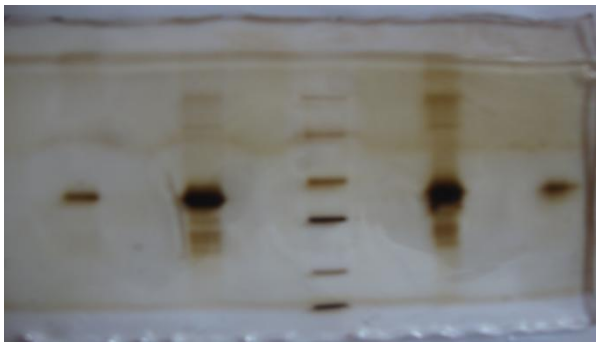
RESULTS

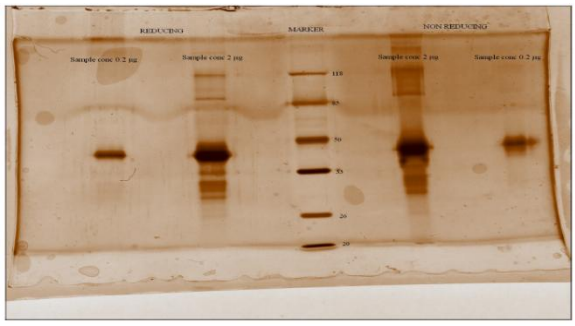
COOMASSIE STAINING



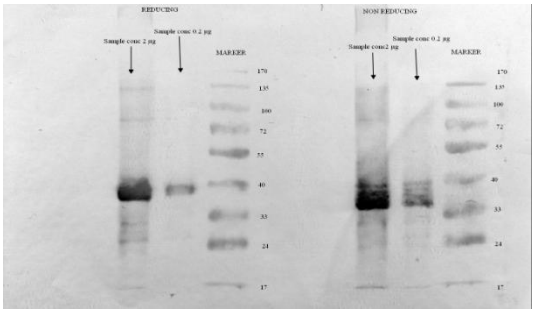


SILVER STAINING





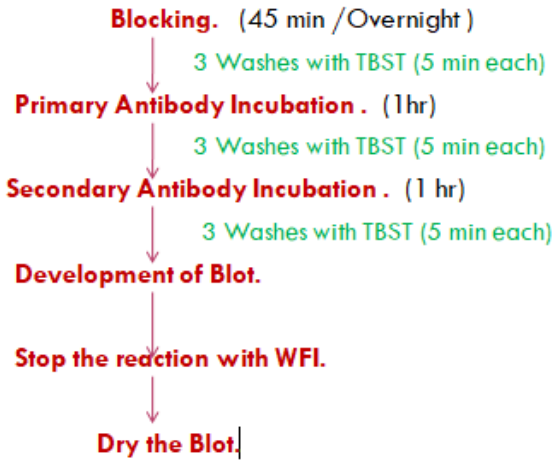
WESTERN BLOTTING



IDENTIFICATION OF VACCINE COMPONENTS BY WESTERN BLOTTING:

- Separate the macromolecules using gel electrophoresis.
- The separated molecules are transferred onto a second matrix, generally a nitrocellulose or polyvinylidene difluoride (PVDF) membrane.

- Electroelution or Electrophoretic transfer because of its speed and transfer efficiency.



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