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STUDIES ON THE EFFECT OF DIFFERENT PRESERVATIVES ON THE QUALITY CONTROL OF ANTITETANIC SERUM

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Abstract

The effects of different preservatives on the keeping quality of antitetanic serum were investigated. The following preservatives were added; cresol 0.3%, cresol 0.4%, phenol 0.5%, phenol 0.25%, phenol 0.5% and merthiolate 1:10,000, merthiolate 1:5000, sodium azide 1:10,000, sodium azide 1:8000, sodium azide 1:5000. Sterility test, total protein, physical appearance, pH values, safety and potency test were studied on each product after one year of storage. In this study, it is proved that the best quality was seen in the antitetanic serum with cresol 0.4% and with sodium azide 1:5000. Both products were clear in appearance, completely sterile from aerobic, anaerobic bacteria, fungus and mycoplasma. They were potent and safe when injected in horses. While, antitetanic serum with phenol 0.5% although it was sterile, potent and safe for injection, it gave turbidity on storage.

INTRODUCTION

Antitetanic serum is a sterile preparation containing immunoglobulins which have the specific power of neutralizing the toxin formed by clostridium tetani. Tetanus antiserum is used in the prophylactic and therapeutic measures against tetanus in animals and man. It was obtained from the blood of healthy horses, firstly immunized by administration of tetanus toxoid and followed by courses of injections of tetanus toxin (Carpenter, 1975).

A suitable antimicrobial preservative must be added to the serum. Merthiolate 0.01% is used (Trinca and Reid, 1967), phenol 0.5% or cresol 0.4% (Merchant and Packer, 1983). Martindale (1996) reported that antisera for human use must not contain phenol more than 0.25%. He also stated that sodium azide is used as a preservative for serum samples.

In this study, different preservatives were added with different concentrations to

improve the production of antitetanic serum and follow their effects on the sterility, physical appearance, total protein, potency and safety of each product.

MATERIALS AND METHODS

I. Materials

1. Experimental animals

a) Horses: i. Ten adult healthy horses aged 3-5 years were used for production of antitetanic serum.
ii. Two groups of horses each of two for safety test.

b) Mice: Ten groups of Swiss mice, each of five (15-20 gm weight).

c) Guinea pigs: Ten groups each of two (300-400 gm weight).

2. Tetanus toxoid

It was supplied by Burroughs Tewlcome Co., London, and standardized to contain 1000 Lf/ml.

3. Tetanus toxin

Purified concentrated tetanus toxin containing 200,000 guinea pigs MLD/ml. was obtained from Egyptian Organization of Biological and Vaccine, Agouza, Giza, Egypt.

4. Phosphate buffer saline (pH 7.2)

It was prepared according to Dulbeco and Voget (1954).

5. Preservatives

a) Phenol: From El-Nasr Pharmaceutical Chemicals Co.

b) Merthiolate: Fluka AG, Buchs SG, Switzerland.

c) Cresol: Obtained from Sigma Chemical Company.

d) Sodium azide: Obtained from Sigma Chemical Company.

6. Spectrophotometer

Beckman DU 7400, USA.

7. Media used for sterility tests

a) Nutrient agar: Code No. CM3-Oxoid, LTD, Basing Stock, Hants, England.

b) Sabaroud dextrose agar: Cat. No. 0110-10-Difco Laboratories, Detroit, Michigan, USA.

c) Thioglucolate broth: Code No. CM 173, Oxoid LTD, Basing stock, Hants, England.

d) PPLO broth: Cat. No. 0554-17-1, difco, USA.

e) Mycoplasma agar: Code No. CM 401, Oxoid, England.

II. Methods

1. Production of antitetanic serum

It was produced according to Merchant and Packer (1983).

2. Types of preservatives added to the antitetanic serum

a) Merthiolate was added in 0.01% to the antitetanic serum according to Trinca and Reid (1967).

b) Merthiolate 0.01% plus phenol 0.5% were added to the antitetanic serum.

c) Phenol was added in 0.5% according to British Pharmacopoeia 1985.

d) Phenol was added in 0.25% according to Martendal (1996).

e) Sodium azide was added in 1:10,000, 1:5000 and 1:8000.

f) Cresol was added in 0.3% (Carpenter, 1975) and in 0.4% according to Merchant and Packer (1983).

3. Evaluation of the antitetanic serum

A. Sterility tests: The sterility tests were conducted according to US Code of Federal Regulations (1987).

B. Determination of pH: pH of the different products was determined by using pH meter.

C. Determination of total protein: It was determined by Spectrophotometer, Beckman, DU 7400, USA.

D. Safety tests

i) Mice: Groups of mice, each of 5, were injected S/C with 0.5 ml from each product and observed for 7 days.

ii) Guinea pigs: Groups of guinea pigs, each of 2, were injected I/P with 2ml of each product and observed for 7 days.

iii) Horses: Two groups of horses each of two were injected subcutaneously; 1st group with 20ml antitetanic serum with cresol 0.4%, and 2nd group with 20ml antitetanic serum with sodium azide 1:5000. These horses were observed seven days.

E. Standardization of the antitetanic serum

Potency of different prepared batches of antitetanic serum with different preservatives were titrated monthly for one year by flocculation test described by Norris and Ribbons (1971).

RESULTS AND DISCUSSION

In this study, different batches of antitetanic serum were prepared and different preservatives were added and their effects followed on the sterility, total protein, physical appearance and pH values, safety and potency of each product after one year of storage.

The following preservatives were applied: phenol 0.5%, phenol 0.25%, phenol 0.5% and merthiolate 1:10,000, merthiolate 1:5000, cresol 0.5%, cresol 0.4%, sodium azide 1:10,000, sodium azide 1:5000 and sodium azide 1:8000.

Keeping quality tests for different batches were studied. Sterility tests according to US Code of Federal Regulations (1987) proved that antitetanic serum for each batch at the first month of storage was sterile in most batches, while, in case of serum preserved with merthiolate 1:10,000, phenol 0.25%, sodium azide 1:10,000 were contaminated. At the end of storage, serum with cresol 0.4%, sodium azide 1:5000 and phenol 0.5% were the only sterile batches as shown in Table 1. These results agreed with Toply and Wilson (1998) who stated that phenol and cresol are bactericidal to Gram positive and Gram negative bacteria and also posses anti-fungal activity.

Cresol acts in much the same way as phenol, but their germicidal activity is usu-

ally somewhat higher, while, merthiolate is said to be bacteriostatic and mildly bactericidal (Toply and Wilson, 1975). Thiomersal is a bacteriostatic and fungistatic mercurial antiseptic used in concentrations 0.01% to 0.02% (Martindale, 1996).

Total protein and pH values were determined in each batch as shown in Table 2. The best result was in serum with cresol 0.4%, sodium azide 1:8000, sodium azide 1:5000. Total protein must not be more than 17 percent w/v (British Pharmacopeia, 1985).

Physical appearance of each batch was recorded in Table 3 indicating that serum with cresol 0.4% and serum with sodium azide 1:5000 gave clear appearance during one year of storage. Merthiolate when added gave precipitate during the first months of storage and thus agreed with Martindale (1996) who stated that merthiolate in slight acid solution may be precipitated and this was proved when merthiolate was added with phenol as preservatives to the serum. 0.5% phenol added to serum gave clear appearance at first months of storage but at the end of storage they became turbid.

Safety test was given in Table 4 and proved that mice and guinea pigs, when injected with different batches except in those with merthiolate, no death occurred during seven days, none of animals showed significant local or systemic reaction according to British Vet. Pharmacopeia (1985).

These results agreed with Cox (1987) who said that severe reactions to vaccines preserved with merthiolate, and also, agreed with Noel (1991) who stated the hypersensitivity to merthiolate in hepatitis B vaccine. Martindale (1996) said that serious adverse effects have followed parenteral and topical use of thiomersal. Antitetanic serum with cresol 0.4% and sodium azide 1:5000 were safe when injected in horses.

Potency of the antitetanic serum in each batch were determined in Table 5. The best products which contained the same international units (IU) along one year of storage were phenol 0.5%, cresol 0.4% and sodium azide 1:5000, while, other batches lost their potency gradually.

Nicholas and Leslie (1988) proved that cresol is more bactericidal but less caustic and less toxic than phenol and inexpensive and efficient as a disinfectant. Olivier *et al.* (1998) reported that sodium azide in concentration of 1/5000 is used as a good preservative to purified serum antibodies. These observations agreed with our results.

On conclusion, cresol 0.4% when added to the antitetanic serum and sodium azide in concentration 1:5000 will give product of good quality.

Table 1. Sterility tests of serum batches with and without different types of preservatives after one and 12 months of storage.

Types of preservatives	Aerobic bacteria		Anaerobic bacteria		Fungus		Mycoplasma	
	1 M	1 Y	1 M	1 Y	1 M	1 Y	1 M	1 Y
1. Control serum	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	- ve	- ve
2. Cresol 0.3%	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
3. Cresol 0.4%	- ve	-ve	- ve	-ve	- ve	-ve	- ve	-ve
4. Phenol 0.5%	- ve	-ve	- ve	-ve	ve	-ve	- ve	-ve
5. Phenol 0.25%	- ve	- ve	- ve	- ve	+ ve	+ ve	- ve	- ve
6. Phenol 0.5% + merthiolate 1:10,000	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
7. Merthiolate 1:10,000	+ ve	+ ve	+ ve	+ ve	- ve	- ve	- ve	- ve
8. Merthiolate 1:5000	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
9. Sodium azide 1:10,000	- ve	-ve	+ ve	+ ve	- ve	- ve	- ve	- ve
10. Sodium azide 1:8000	- ve	+ve	- ve	-ve	- ve	-ve	- ve	-ve
11. Sodium azide 1:5000	- ve	-ve	- ve	-ve	- ve	-ve	- ve	-ve

1 M : One Month. 1 Y : One Year.

Table 2. Total protein content and pH values in the antitetanic serum with and without different preservatives.

Serum with and without preservatives	Total protein (gm/dl)	pH value
1. Control serum (without preservative)	5.8	7.7
2. Cresol 0.3%	5.5	7.8
3. Cresol 0.4%	3.8	8.1
4. Phenol 0.5%	3.7	7.8
5. Phenol 0.25%	3.5	7.5
6. Phenol 0.5% + merthiolate 1:10,000	2.7	7.6
7. Merthiolate 1:10,000	5.2	7.2
8. Merthiolate 1:5000	3.4	7.4
9. Sodium azide 1:10,000	3.9	7.5
10. Sodium azide 1:8000	4.7	7.6
11. Sodium azide 1:5000	4.3	7.7

pH of crude serum ranges from 7-8 gm/dl according to British Pharmacopeia (1985).

Table 3. Physical appearance of different serum batches after one year of storage.

Types of preservatives	Physical appearance
1. Control serum (without preservative)	Turbid
2. Cresol 0.3%	Precipitated
3. Cresol 0.4%	Clear
4. Phenol 0.5%	Turbid
5. Phenol 0.25%	Turbid
6. Phenol 0.5% + merthiolate 1:10,000	Precipitated
7. Merthiolate 1:10,000	Very turbid
8. Merthiolate 1:5000	Precipitated
9. Sodium azide 1:10,000	Precipitated
10. Sodium azide 1:8000	Clear
11. Sodium azide 1:5000	Very clear

Table 4. Safety tests just after addition of different preservatives.

Types of preservatives	Guinea pigs	Mice	Horse
1. Cresol 0.3%	Alive	Alive	ND
2. Cresol 0.4%	Alive	Alive	Alive
3. Phenol 0.5%	Alive	Alive	ND
4. Phenol 0.25%	Alive	Alive	ND
5. Phenol 0.5% + merthiolate 1:10,000	Dead	Dead	ND
6. Merthiolate 1:10,000	Dead	Dead	ND
7. Merthiolate 1:5000	Dead	Dead	ND
8. Sodium azide 1:10,000	Alive	Alive	ND
9. Sodium azide 1:8000	Alive	Alive	ND
10. Sodium azide 1:5000	Alive	Alive	Alive

ND : Not Done.

Table 5. Potency of the antitetanic serum with different preservatives as determined by Flocculation test (FT) and expressed in IU/ml during one year of storage.

Serum with different preservatives	Months during one year of storage											
	1	2	3	4	5	6	7	8	9	10	11	12
1. Cresol 0.3%	200	200	200	200	200	200	190	190	180	170	160	160
2. Cresol 0.4%	200	200	200	200	200	200	200	200	200	200	200	200
3. Phenol 0.5%	200	200	200	200	200	200	200	200	200	200	200	200
4. Phenol 0.25%	200	200	200	200	150	100	80	50	30	-	-	-
5. Phenol 0.5% + merthiolate 1:10,000	90	60	20	20	-	-	-	-	-	-	-	-
6. Merthiolate 1:10,000	120	60	30	30	10	-	-	-	-	-	-	-
7. Merthiolate 1:5000	200	200	200	200	200	190	160	120	160	80	60	60
8. Sodium azide 1:10,000	200	200	200	200	200	200	200	190	160	150	120	100
9. Sodium azide 1:8000	200	200	200	200	200	200	200	200	200	200	200	200
10. Sodium azide 1:5000	200	200	200	200	200	200	200	200	200	200	200	200

IU : International Unit.

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دراسات على تأثير المواد الحافظة المختلفة على الجودة النوعية للمصل المضاد للتيتانوس

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تمت دراسة تأثير المواد الحافظة المختلفة على الجودة النوعية للمصل المضاد للتيتانوس واستخدمت المواد الآتية: كريزول ٠,٣٪ وكريزول ٠,٤٪ وفينول ٠,٥٪ وفينول ٠,٢٥٪ وأيضاً فينول ٠,٥٪ مع ميرثيولات ١:١,٠٠٠ وأُستخدم أيضاً ميرثيولات ١:١,٠٠٠ و١:٥,٠٠٠ وأُستخدم الصوديوم أزيد بنسبة ١:١٠,٠٠٠ ، ١:٨,٠٠٠ ، ١:٥,٠٠٠ .

تمت دراسة اختبار النقاوة وقياس البروتين الكلى والمظهر الطبيعى وقياس درجة الهيدروجين (pH) وكذلك اختبار الأمان لكل منتج بعد سنة من التخزين.

أثبتت الدراسة أن أحسن جودة نوعية كانت فى المصل المضاد للتيتانوس المحتوى على كريزول ٠,٤٪، وكذلك المصل المحتوى على الصوديوم أزيد ١:٥,٠٠٠ . وكل منهما كان رائق المظهر وعلى درجة نقاوة عالية خالياً من الميكروبات الهوائية واللاهوائية والفطريات وكذلك الميكوبلازما وكل محتفظ بالقوة العيارية وكذلك ممكن حقنه بأمان فى الخيول بينما المصل المضاد للتيتانوس والمحتوى على فينول ٠,٥٪ بالرغم أنه كان على درجة نقاوة وكفاءة عيارية وأمان فى الحقن إلا أنه فى نهاية فترة التخزين أعطى مظهراً متعكراً.