Liquor Antisepticus N. F. VI

*By C. O. Beebe, L. W. Busse and A. H. Uhl**

The present formula for Liquor Antisepticus in our opinion and the opinion of others is not as satisfactory as it might be. That it seems to have met with wide disapproval is attested by the following comments, which were received by the sub-committees of the N. F. Revision Committee shortly after the publication of the N. F. VI.

E. FULLERTON COOK: "I have had a number of complaints concerning the tendency of the new Liquor Antisepticus to become cloudy within a few hours, and also the unpleasant taste of the new solutions containing chlorthymol."

BERNARD FANTUS: "Having committed ourselves to the inclusion of chlorthymol and eucalyptol in Liquor Antisepticus would suggest that the maximum amount of these ingredients that remain in solution be specified in the formula."

S. L. HILTON: "If incompatible, delete chlorthymol."

H. R. WEMPLE: "No question about their incompatibility causing cloudiness to appear within a few hours."

After preparing several batches of Liquor Antisepticus and noting the incompatibilities referred to above, we carried out the following experiments in an attempt to improve the formula.

EXPERIMENTAL

These experiments consisted of a comparison of the antiseptic value of Liquor Antisepticus N. F. VI and modifications of it as directed by the general plan of disinfectant testing by the Hygienic Laboratory Method.

A test culture of *Bacillus coli* was used in place of *Bacillus typhosus*, Hopkins strain, as specified by the Hygienic Laboratory Method. Between periods of testing the culture was kept on nutrient agar slants, transferred at monthly intervals.

Phenol meeting the U. S. P. requirements was used in all experiments.

A 5% original solution was made by adding one part by weight of phenol, liquefied by warming the bottle, to nineteen parts of distilled water. A fresh solution was used for each day's testing.

Dilutions of phenol and disinfectant were made from the original liquid on the day of the test. The dilutions of phenol used were 1:80, 1:90, 1:100, 1:110, 1:120; 5 cc. of each was placed in a seeding tube, 1 x 3 inches with a flared top and a round bottom.

Dilutions of antiseptic solution used were, full strength, 1:2, 1:3, 1:4, 1:5; 5 cc. of each were placed in a seeding tube. All dilutions were made with sterile distilled water.

To test each tube, 0.1 cc. of culture was added seriatim, allowing 15 seconds for each addition. Five dilutions of antiseptic solution and 5 dilutions of phenol were used which required $2^1/_2$ minutes to carry out the procedure.

At the end of five minutes from the time the culture was added to the seeding tube, a mixture was transferred from the tube to a subculture tube and this procedure was carried out for each successive seeding tube at 15-second intervals. This procedure was repeated at $7^1/_2$, 10, $12^1/_2$ and 15 minutes from the time of the first addition of the culture to the seeding tube.

The tubes were properly labeled and placed in the incubator for 48 hours at 37° C., at the end of which time readings of growth or no growth were made and entered in a table as + or 0 signs, respectively.

The coefficient is the arithmetic mean of the sums of three ratios, expressed decimally. These ratios are the denominator of the highest dilution of the disinfectant in the subculture tube of which no growth occurs, divided by the corresponding figure for phenol, for the 5, 10 and 15-minute intervals, respectively.

Liquor Antisepticus and seven different modifications of it were prepared and tested. The modifications consisted solely of the deletion of one of the ingredients in an effort to determine what effect the various ingredients exerted upon the antiseptic properties of the solution. The following table is a summary of our results:

TABLE I.—EFFECT OF DELETING INGREDIENTS ON PHENOL COEFFICIENT

Expt. No.	Antiseptic Solution	Ingredient Deleted	Phenol Coefficient
1	N. F. VI	.....	0.023
2	N. F. VI	Boric acid	0.023
3	N. F. VI	Thymol	0.020
4	N. F. VI	Chlorthymol	0.012
5	N. F. VI	Menthol	0.036
6	N. F. VI	Eucalyptol	0.042
7	N. F. VI	Methyl salicylate	0.037
8	N. F. VI	Oil of thyme	0.027

From the above table we can draw the following conclusions:

1. Boric acid has no effect on the antiseptic value of the N. F. Antiseptic Solution.

2. The absence of either thymol or chlorthymol decreases the antiseptic value of the solution, the chlorthymol causing the greatest reduction.

3. Without menthol, eucalyptol, methyl salicylate or oil of thyme, N. F. Antiseptic Solution has a

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TABLE II.—EFFECTS OF DECREASED AMOUNTS OF THYMOL, CHLORTHYMOL AND MENTHOL

Solution	Boric Acid, Gm.	Thymol, Gm.	Chlor-thymol, Gm.	Menthol, Gm.	Eucalyp-tol, Cc.	Methyl Salicyl-ate, Cc.	Oil of Thyme, Cc.	Alcohol, Cc.	Phenol Coeffi-cient
N. F. Bulletin Antisep-tic Soln. Formula	25.00	0.5	0.5	0.5	0.2	1.2	0.3	250	0.049
Expt. 10	10.00	0.5	0.5	0.5	0.2	1.2	0.3	300	0.049
Expt. 11	25.00	1.0	...	0.5	0.2	1.2	0.3	250	0.024

TABLE III.—MAXIMUM QUANTITIES OF INGREDIENTS AND TEMPERATURES AT WHICH CLEAR SOLUTION RESULTS

Solu-tion No.	Boric Acid, Gm.	Thymol, Gm.	Chlor-thymol, Gm.	Men-thol, Gm.	Eucalyp-tol, Cc.	Methyl Sali-cylate, Cc.	Oil of Thyme, Cc.	Alco-hol, Cc.	Temp. Cloudy, ° C.	Phenol Coeffi-cient
1	25.00	0.5	0.5	0.5	0.19	0.12	0.03	250.00	33	...
2	20.00	...	...	...	0.2	1.2	0.3	300.00	32	...
3	...	...	...	...	0.2	1.2	0.3	300.00	32	...
4	...	...	1.0	...	0.1	1.0	...	300.00	31	...
5	25.00	...	0.8	...	0.1	0.4	0.1	300.00	20	...
6	25.00	...	0.75	0.5	...	1.0	...	300.00	30	...
7	20.00	...	1.00	...	...	1.0	...	300.00	24	...
8	20.00	...	0.75	...	...	1.0	...	300.00	16	0.072
9	25.00	...	0.75	...	0.19	0.12	0.03	300.00	11	...
10	25.00	...	0.70	0.1	0.15	0.15	0.05	300.00	14	...
11	25.00	...	0.70	0.15	0.15	0.15	0.05	300.00	18	0.072
12	25.00	0.4	0.4	0.4	...	1.2	0.3	300.00	38	...

greater antiseptic value, the preparation without eucalyptol having the highest phenol coefficient.

After the effects of the deletion of the various ingredients of the formula on the phenol coefficient had been determined, our experiments were directed toward arriving at a formula which did not have the incompatibilities of the present formula, and which had as high or higher antiseptic value. Our efforts in solving the problem were directed toward using decreased amounts of the troublesome ingredients.

The effects of decreased amounts of thymol, chlorthymol and menthol are shown in Table II.

Decreasing the amounts of the chlorthymol and menthol from 1 Gm. to 0.5 Gm. and eucalyptol from 2 cc. to 0.2 cc. increased the antiseptic value of the solution. The solution, however, was cloudy and had to be clarified.

Reducing the boric acid to 10 Gm. and increasing the alcohol 50 cc. per L. gave a cloudy preparation which when clarified had no apparent effect on its antiseptic value.

Substituting 1 Gm. of thymol for 0.5 Gm. each of chlorthymol and thymol in Experiment 11 produced a cloudy solution which when clarified was of less antiseptic value than the former preparation.

Further results in the preparation of solutions, using decreased amounts of the various ingredients, are shown in Table III.

By comparing the amounts of the ingredients in the various preparations and noting the temperature at which they become cloudy, one arrives at the following conclusion:

1. By comparing solutions 2 and 3, it is seen that at room temperature a clear solution cannot be prepared using eucalyptol 0.2 cc., methyl salicylate 1.2 cc. and oil of thyme 0.3 cc. The temperature at which the product was clear was 34° C.

2. The reduction of eucalyptol to 0.1 cc., methyl salicylate to 0.4 cc., oil of thyme to 0.1 cc., using 0.8 Gm. of chlorthymol, 25 Gm. of boric acid and 300 cc. of alcohol, produced a clear solution at 22° C.

3. By comparing solutions number 9, 10, 11 and 12 it will be noted that the maximum amounts of ingredients giving a clear solution at 19° C. are those represented by solution 11.

GENERAL SUMMARY

1. N. F. Antiseptic Solution, before using purified talc to clarify the product, is a cloudy, milky appearing solution. The deletion of any one ingredient has little beneficial effect.

2. Boric acid exhibits no effect upon the antiseptic value (phenol coefficient) of N. F Antiseptic Solution.

3. A higher antiseptic value, as determined by its phenol coefficient, will result upon the reduction of the amounts of volatile oils used in the preparation of N. F. Antiseptic Solution.

4. If a solution is to be prepared which will remain clear at ordinary temperatures without clarification, only certain amounts

of the ingredients in N. F. Antiseptic Solution can be used.

5. When decreased amounts of the ingredients in N. F. Antiseptic Solution are used, providing chlorthymol is not less than 0.5 Gm. per liter, the antiseptic value is equal to that of the present official solution.

The following formula with working directions is offered for criticism. This solution when submitted to the test for antiseptic value, described for N. F. Antiseptic Solution, showed *Staphylococcus aureus* to be killed in $^1/_2$ minute as shown by no growth on the subculture after incubation for 48 hours at 37.5° C.

Boric acid	25.00 Gm.
Chlorthymol	0.7 Gm.
Menthol	0.15 Gm.
Methyl salicylate	0.15 Gm.
Eucalyptol	0.15 Gm.
Oil of thyme	0.05 cc.
Alcohol	300.00 cc.
Distilled water *q. s.*	
To make	1000.00 cc.

Dissolve the boric acid in 650 cc. of warm distilled water and the other ingredients in the alcohol. Cool the boric acid solution, add it to the alcoholic solution with continual agitation; finally add sufficient distilled water to make 1000 cc. and mix well.

Assay of Sarsaparilla Preparations*

Preliminary Report

By B. Fantus, M.D., and H. A. Dyniewicz, Ph.C.

It is the consensus of modern medical opinion that the compound syrup of sarsaparilla is chiefly a flavoring vehicle (U. S. Dispensatory, page 1069, 21st Edition). This opinion was responsible for the deletion of the Fluidextract of Senna from the formula in the tenth revision as laxative effect might not be wanted in a mere flavoring agent. It may, indeed, be questioned whether the sarsaparilla might not be deleted from the formula, for the pleasant flavoring qualities of this syrup are not due to the sarsaparilla at all but are due to the volatile oils contained in the preparation. Indeed, what is by common consent called the sarsaparilla flavor is really a "sassafras bouquet," and the title "Compound Syrup of Sassafras" would be preferable unless it can be shown that sarsaparilla has some pharmacologic and possibly therapeutic action.

The question is whether the presence of the sarsaparilla is of any advantage in the compound syrup.

Prof. Gathercoal's prescription survey (1) shows the usage of the Compound Syrup of Sarsaparilla has been 60 per 10,000 prescriptions for the last 30 years. With this considerable usage in mind, in spite of the unfavorable opinion of the pharmacologists as to any medicinal value of sarsaparilla, we undertook this study in an endeavor to find upon what effect, if any, the preparations may be assayed.

According to a report by F. W. Apt (2) sarsaparilla is dependent upon saponin for its activity, and the saponin increases absorption of the medicament that may be carried in a preparation of sarsaparilla. The U. S. Dispensatory (21st Edition, page 963) reads: "The sarsaparilla of commerce is apt to be nearly, if not quite inert, either from age or from having been obtained from inferior species. The only criterion of good sarsaparilla to be relied on is its taste. As it leaves a decided acrid impression in the mouth it is considered effective; otherwise it is inert."

There are in the literature numerous references on the increased absorption of various drugs by means of saponins as will be seen by consulting the bibliography (3 to 10). Hence the sarsaparilla assay should be for saponin.

According to R. Kobert (11) the activity of the saponins is best evaluated by the hemolysis of red blood cells in physiologic salt solution in a given time. This was substantiated by C. Sormani and J. Rühle (12) and K. Hering (13). A test based upon hemolysis was developed by these investigators and modified by W. Brandt (14). A table of 80 drugs giving

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