

Basic Clinical Trial Design and Considerations for Conducting On-Farm Evaluations of Complementary Therapy



Ynte Schukken and Linda Tikofsky

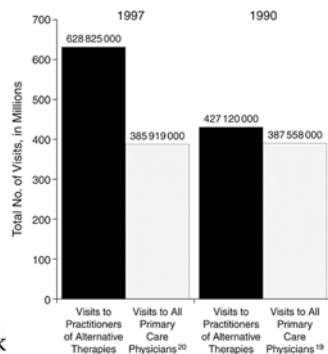
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Complementary and alternative therapies

- There is an increased interest in alternative treatments for disease in dairy cows.
- Scientific evidence for many alternative treatments is quite limited.
- Many treatment studies originate on commercial dairy farms.
- Relative simple principles lead to valid studies



Trends in annual visits to practitioners of alternative therapies vs visits to primary care physicians, United States, 1997 vs 1990



Fisenberg, D. M. et al. JAMA 1998;280:1568-1575

JAMA

A trial was conducted to test the efficacy of a new treatment for coliform mastitis.

Of 50 coliform infected cows treated, 80% were free of the mammary infection by 2 weeks post treatment.

What is your conclusion ?

“Results show that 80% of the cases of a starting udder inflammation can be successfully treated with XXX Mint Spray.”

From Simmer-Agri website



- An investigation was undertaken to evaluate the effectiveness of a homeopathic complex in the management of clinical mastitis.
- A total of 102 mastitic quarters were treated with a homeopathic complex consisting of Phytolacca 200c, Calcarea fluorica 200c, Silicea 30c, Belladonna 30c, Bryonia 30c, Arnica 30c, Conium 30c and Ipecacuanha 30c.
- The diagnosis of mastitis and the recovery criterion was based on physical examination of udder, milk and CMT score.
- Treatment was 80 to 96.72% effective.

Any concerns with the validity of this study ?



Varshney JP, Naresh R. Evaluation of a homeopathic complex in the clinical management of udder diseases of riverine buffaloes. Homeopathy. 2004 Jan;93(1):17-20.

Another Mastitis treatment trial:

- Cows considered to be valuable by the owners were excluded from the trial.
- Cows were assigned to a treatment based on the result of a coin-flip.

Any concerns with the validity of this study ?



Quality of Evidence and Grades of Recommendations

Grade of recommendation	Study design
A	Systematic review of randomized controlled trials
	Individual randomized controlled trial
B	Systematic review of cohort studies
	Individual cohort study
C	Case series
D	Expert opinion without explicit critical appraisal or based on physiology or bench research



Case series

- Description of a series of cases
- Initial description of 'new' treatment
- Data quality important issue:
 - Objective measure of cure
 - Consistency of treatment use
- Causal conclusions are limited
- Examples:
 - Uddermint
 - Homeopathic treatment study in Buffalo



Cohort study

- Animals that are treated with treatment A are compared to animals that are treated with treatment B.
- 'Comparative case series'
- No randomization
- Cohorts may be different with regard to important predictive factors (i.e age, sickness score etc.).
- Example:
 - Compare 20 cases of mastitis treated with a new treatment to 20 cases of mastitis treated with the usual treatment on the same farm in the year before.



Randomized controlled trial

- Animals are randomized to either treatment or control group.
- Treatment and control occur on the same farm during the same time period.
- Treatment and control group are identical with regard to important predictive factors.
- The animals are evaluated in an unbiased manner.
- Example:



Original Article

Comparison of Homeopathy, Placebo and Antibiotic Treatment of Clinical Mastitis in Dairy Cows – Methodological Issues and Results from a Randomized-clinical Trial

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Summary

Go to section

Based on the widespread use of homeopathy in treatment of animal disease and the poor documentation of its possible effects and consequences, a clinical trial was carried out in order to evaluate the efficacy of homeopathy in treatment of clinical mastitis in dairy cows and a design for clinical studies on homeopathic treatment, taking into account the guidelines for randomized-clinical trials (RCT) as well as the basic principles of homeopathy. A three-armed, stratified, semi-crossover design comparing homeopathy, placebo and a standardized antibiotic treatment was used. Fifty-seven dairy cows were included. Evaluation was made by two score scales, with score I measuring acute symptoms and score II measuring chronic symptoms, and by recording the frequencies of responders to treatment based on four different responder definitions. Significant reductions in mastitis signs were observed in all treatment groups.



Quality of Clinical Evidence and Grades of Recommendations

Grade of recommendation	Study design
A	Systematic review of randomized controlled trials
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Non-randomized Trials May Be Appropriate

- Early studies of new and untried therapies
- Uncontrolled early phase studies where the standard therapy is relatively ineffective
- Truly dramatic response



But this is the exception !

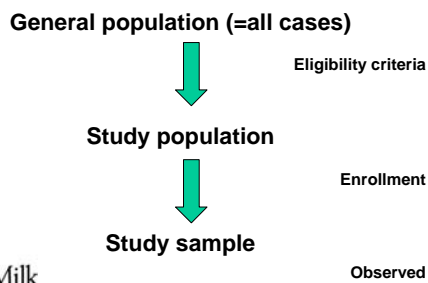
Relative simple principles lead to valid clinical studies

- 1) Select relevant cases
- 2) Use a contemporary control group.
- 3) Randomly assign treatment to all animals in the study.
- 4) Use sufficient cases
- 5) Use objective evaluation methods where this is possible.



Study Population

Subset of the general population determined by the eligibility criteria



Comparing Treatments with a relevant control

- Use of contemporary controls
 - Groups must be alike in all important aspects and only differ in the intervention each group receives
 - In practical terms, "comparable treatment groups" means "alike on the average"
- Methods to make treatment groups more alike:
 - Large numbers
 - Stratification
 - Stratify by important predictors of outcome
 - Herd, age, previous disease, severity etc.
 - Paired studies



Stratification variable

Stratification factors	Number of patients	Initial Score I	% Reduction of Score I, Day 7
First lactation	10	17.8 (15.3-20.3)	30.1 (16.9-43.2)
Second or higher lactations	47	18.7 (17.6-19.7)	34.0 (28.0-40.1)
Mild mastitis	15	15.7* (14.6-16.8)	27.9* (17.5-38.3)
Moderate mastitis	30	17.8* (17.0-18.6)	33.6 (26.2-40.9)
Severe mastitis	12	23.8* (22.5-25.0)	40.9* (29.3-52.5)
Negative	13	15.2* (13.3-17.2)	38.4 (29.5-47.3)
<i>S. aureus</i>	19	19.7 (18.2-21.1)	28.9 (19.9-38.0)
Others	22	19.3 (18.0-20.6)	37.6 (28.7-46.5)
<i>Escherichia coli</i>	3	19.3 (15.7-23.0)	13.7 (-17.4-44.8)



Advantages of Randomization

1. Randomization "tends" to produce comparable groups
2. Randomization produces valid statistical tests
3. Randomization should be truly random and verifiable (flipping coins ?)



Sample Size

- Sample size is an estimate, using guidelines and assumptions.
- Depends on input of:
 - Type I error (usually 5%)
 - Type II error (usually ~ 20%)
 - Size of difference to be detected
 - ‘Showing’ difference vs. showing equivalence



Errors in Hypothesis Testing

Null: New treatment and control are equivalent
 Alternative: New treatment is better than control

Decision	Truth	
	Null hypothesis	Alternative hypothesis
Do not reject null	OK	TYPE II ERROR
Reject null	TYPE I ERROR	OK

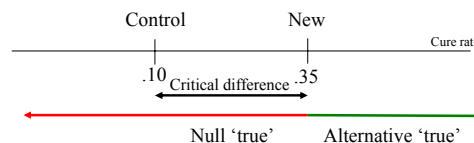


Definitions: Types of Errors

- Type I error: The null hypothesis is rejected when it is true.
- Type II error: The null hypothesis is not rejected when it is false.
- There is always a chance of making one of these errors. We'll want to minimize the chance of doing so!



Difference between 2 treatments



Null: New treatment and control are equivalent
 Alternative: New treatment is better than control

Note: single sided test !

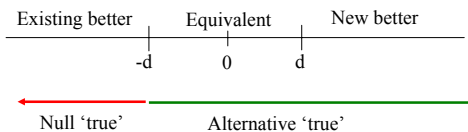


Sample size calculator



<http://home.clara.net/sisa/>

Equivalence of 2 treatments

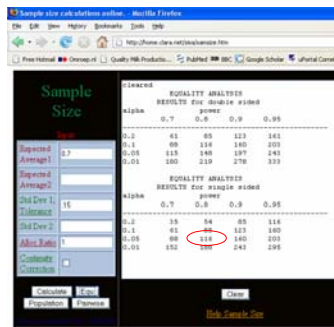


$$H_0: \text{New is worse than existing} = P_{\text{New}} - P_{\text{Existing}} \leq -d$$

$$H_A: \text{New is equivalent or better} = P_{\text{New}} - P_{\text{Existing}} > -d$$



Sample size calculation



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Ok, if you did not get this...
go on a date with a statistician:



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From: Gonick and Smith, Cartoon Guide to Statistics

Objective evaluation methods

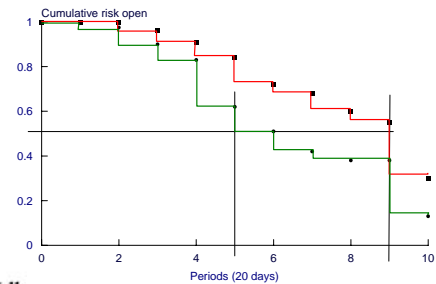
- Objective methods:
 - SCC, Culture, Conception

Pre-treatment		Post-treatment		Interpretation
Day 0	Day 14	Day 21	Bacteriology	
S. aureus	S. aureus	S. aureus	Fail	Fail
S. aureus	S. aureus	Negative	Fail	Fail
S. aureus	S. uberis	S. uberis	Cure	Cure
S. aureus	Negative	Negative	Cure	Cure
Negative	S. uberis	S. uberis	Not eligible	Not eligible
Negative	Negative	Negative	Not eligible	Not eligible

- Subjective methods → Blinding essential

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Time to conception



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So, relative simple principles lead to
valid clinical studies:

- 1) Select relevant cases
- 2) Use a contemporary control group.
- 3) Randomly assign treatment to all animals in the study.
- 4) Use sufficient cases
- 5) Use objective evaluation methods where this is possible.

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Example Trial Design Orbeseal

1. 2 groups: DCT , DCT+Orbeseal
2. Lactation 1 and higher, clinically healthy, no contagious mastitis, not treated in last 30 days, 4 functional quarters, dry-period 30-70 days.
3. Formal randomization using randomization form and envelopes.
4. Blinding: different person treating and diagnosing mastitis after calving
5. Samples size ~200 cows per group
6. Outcome: New infections, Clinical mastitis.

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Sample size

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J. Dairy Sci. 86:3899-3911
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Effectiveness of an Internal Teat Seal in the Prevention of New Intramammary Infections During the Dry and Early-Lactation Periods in Dairy Cows when used with a Dry Cow Intramammary Antibiotic

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Table 4. Results of multivariate regression analysis of odds for presence of an IMI at 1 to 3 DIM and odds of acquiring a new IMI between dry off and 1 to 3 DIM for control quarters (DCT) and treated quarters (DCT plus Orbeseal).¹

Parameter of interest	Control (n = 812) n affected (%)	Treated (n = 821) n affected (%)	Estimate (SE)	Odds ratio _{treated/control} (95% Conf. limits)	P value
IMI present at 1 to 3 DIM					
All pathogens	296 (29.1%)	187 (22.8%)	-0.37 (0.11)	0.69 (0.56, 0.85)	0.008
Major pathogens	149 (18.3%)	116 (14.1%)	-0.36 (0.13)	0.70 (0.54, 0.91)	0.009
Minor pathogens	73 (9.0%)	51 (6.2%)	-0.41 (0.16)	0.66 (0.46, 0.97)	0.03
Contagious pathogens	19 (2.3%)	14 (1.7%)	-0.28 (0.34)	0.76 (0.39, 1.47)	0.42
Environmental streptococci	58 (10.2%)	61 (7.4%)	-0.38 (0.17)	0.68 (0.48, 0.95)	0.02
Gram Negative pathogens	75 (9.2%)	70 (8.5%)	-0.14 (0.16)	0.87 (0.63, 1.20)	0.40
New IMI acquired between dry off and 1 to 3 DIM					
All pathogens	206 (25.4%)	168 (20.2%)	-0.39 (0.11)	0.70 (0.56, 0.87)	0.002
Major pathogens	135 (16.6%)	107 (13.0%)	-0.25 (0.14)	0.71 (0.54, 0.93)	0.01
Minor pathogens	58 (7.1%)	42 (5.1%)	-0.37 (0.22)	0.69 (0.45, 1.05)	0.09
Contagious pathogens	19 (1.2%)	8 (1.0%)	-0.53 (0.41)	0.59 (0.28, 1.25)	0.20
Environmental streptococci	81 (10.0%)	60 (7.3%)	-0.39 (0.17)	0.68 (0.48, 0.95)	0.02
Gram-negative pathogens	71 (8.7%)	66 (8.0%)	-0.14 (0.17)	0.87 (0.63, 1.21)	0.41

¹Models control for herd, parity, month, linear score, and infection status at dry off.

Table 5. Results of multivariate regression analysis of odds for experiencing a clinical mastitis event between dry off and 60 DIM for control quarters (DCT) and treated quarters (DCT plus Orbeseal).¹

Pathogen cultured	Control (n = 802) n affected (%)	Treatment (n = 802) n affected (%)	Estimate (SE)	Odds ratio _{treatment/control} (95% Conf. limits)	P value
All pathogens	69 (8.6%)	51 (6.3%)	-0.40 (0.17)	0.67 (0.48, 0.93)	0.02
Major pathogens	32 (3.7%)	19 (2.3%)	-0.73 (0.26)	0.48 (0.28, 0.85)	0.008
Minor pathogens	8 (1.0%)	9 (1.0%)	-0.02 (0.44)	0.98 (0.42, 2.30)	0.98
Contagious pathogens	3 (0.4%)	3 (0.4%)	0.006 (0.70)	1.01 (0.25, 4.0)	0.99
Environmental streptococci	17 (2.0%)	3 (0.4%)	-1.79 (0.68)	0.17 (0.04, 0.62)	0.0069
Gram Negative pathogens	12 (1.4%)	13 (1.5%)	-0.057 (0.41)	0.94 (0.43, 2.10)	0.89

¹Models control for herd, days dry, and parity, month, linear score, and infection status at dry off.

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Orbeseal study

- Well designed
- Large sample size
 - [independence of quarters is an issue]
- Objective outcome: culture
- Subjective outcome: mastitis
 - Blinded investigator

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Example 2 Hektoen et al. 2004

- A randomized, observer-blinded and placebo-controlled trial.
- A stratified, modified three-dimensional semi-crossover design.
 - In this study, a modified semi-crossover design was used, crossing non-responders in the homeopathy and placebo groups to antibiotic treatment and non-responders in the antibiotic group to homeopathic treatment.

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Example Hektoen et al. 2004

- A trained homeopath examined the cows in the homeopathy and placebo groups, selected the homeopathic remedy and initiated treatment (or placebo) on day 0. Follow-up treatments were given by the farmers.
- The homeopath was in contact with the farmers by phone and re-examined the cow if necessary.
- Change of homeopathic remedy within the same set was allowed as long as the cow was not classified as a non-responder to treatment and crossed over to a new treatment group.

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Sample size

- In order to detect a difference between the treatments of one times the SD of the outcome measurement, with a power of 90% and a significance level of 5%:
 - at least 18 patients in each group had to be included.



Outcome scoring

- Score I was used to measure acute changes. This score scale included
 - Body temperature,
 - Appetite,
 - Acute inflammation symptoms in the affected quarter,
 - Visible changes in milk,
 - California Mastitis Test (CMT)
 - Bacteriological findings.
- Score II was used to measure chronic changes. This score scale included
 - Atrophy,
 - Fibrosis
 - Milk production in the affected quarter
 - Visible changes in milk,
 - California Mastitis Test (CMT)
 - Bacteriological findings.
- Each measure was scored on a scale from 1 to 5, scores were added for each of the two score-scales. Both score scales had a range from 6 to 30.



Disease scoring system

Score factors	Score values				
	1	2	3	4	5
Score I					
Temperature °C	38.0–39.2	*	39.3–39.5	39.6–40.0	>40.0
Appetite	Normal	*	Slightly reduced	Reduced	None
inflammation signs	None	*	Moderate	Severe	Very severe
Changes in milk	None	*	Moderate	Severe	Very severe
CMT	1	2	3	4	5
Bacteria in milk	Negative	*	Positive/different from inclusion	*	Positive/same as at inclusion

*Value not used.



Continuous scales (rather than binary) have the advantage of greater power.

Disease scoring system

Score factors	Score values				
	1	2	3	4	5
Score II					
Atrophy	None	*	Moderate	Severe	Very severe
Fibrosis	None	*	Moderate	Severe	Very severe
Changes in milk	None	*	Moderate	Severe	Very severe
Milk production in affected quarter	Normal	*	Slightly reduced	Reduced	Dry quarter
CMT	1	2	3	4	5
Bacteria in milk	Negative	*	Positive/different from inclusion	*	Positive/same as at inclusion

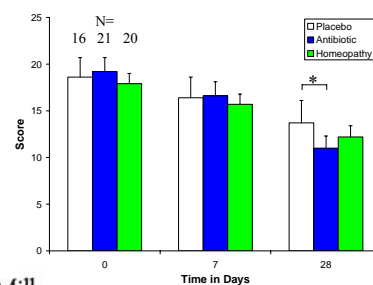
*Value not used.



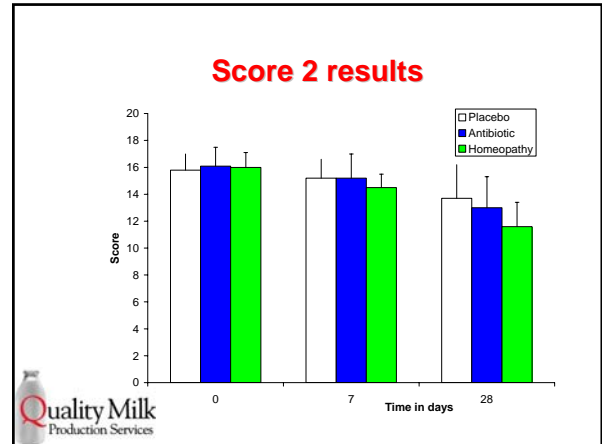
Treatment	Score I			
	Day 0	Day 1	Day 7	Percentage reduction day 7
Homeopathy (n = 21)	17.9 (16.7–19.0)	15.7 (14.5–16.8)	12.2 (10.1–14.4)	31.0 (22.5–39.6)
Placebo (n = 16)	18.6 (16.5–20.6)	16.4 (14.1–18.6)	13.7 (11.3–16.1)	26.6 (16.8–36.3)
Antibiotic (n = 20)	19.2 (17.6–20.7)	16.6 (15.1–18.1)	11.0 (9.7–12.2)	42.0 (33.3–50.7)



Score 1 results



Treatment	Score II			Percentage reduction day 28
	Day 0	Day 7	Day 28	
Homeopathy (n = 21)	16.0 (14.9–17.1)	14.5 (13.5–5.5)	11.6 (9.8–13.4)	27.6 (17.6–37.6)
Placebo (n = 16)	15.8 (14.4–17.1)	15.2 (13.4–17.0)	13.7 (11.4–16.0)	12.9 (0.06–25.7)
Antibiotic (n = 20)	16.1 (14.9–17.2)	15.2 (13.8–16.7)	13.0 (10.5–15.5)	20.1 (7.8–32.4)



- ### Placebo-controlled mastitis study
- **Complex design**
 - Adequate control groups
 - Allows for homeopathic treatments
 - **No significant difference → Power calculation:**
 - the study had an actual power of 58% to detect the observed difference between homeopathy and placebo.
 - To detect the observed difference between these two groups with a power of 80% at the 95% significance level, at least 31 patients in each group are needed. [note: 3 vs 2 treatment groups].



- ### Example *S.aureus* treatment trial Tikofsky and Zadoks 2007
- Randomized controlled block design.
 - Samples size estimation based on 10% cure in control and 35% cure in treated. Chose 2:1 ratio of treated vs. control.
 - Inclusion and Cure based on:
 - Bacteriology (3x before trt, 3x after trt)
 - SCC
 - Strain typing of isolates.
 - Controls were not treated (=not blind).
 - Treatment:



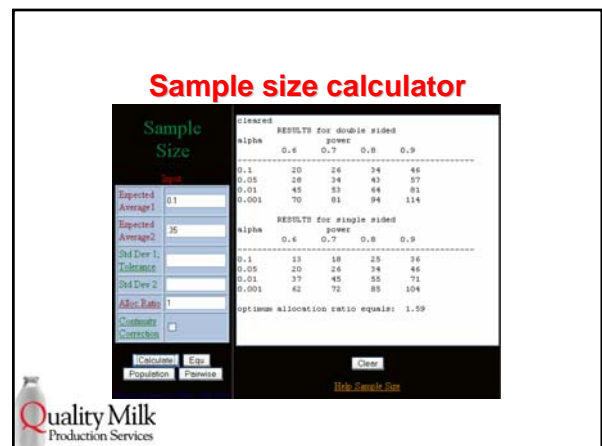
Treatment

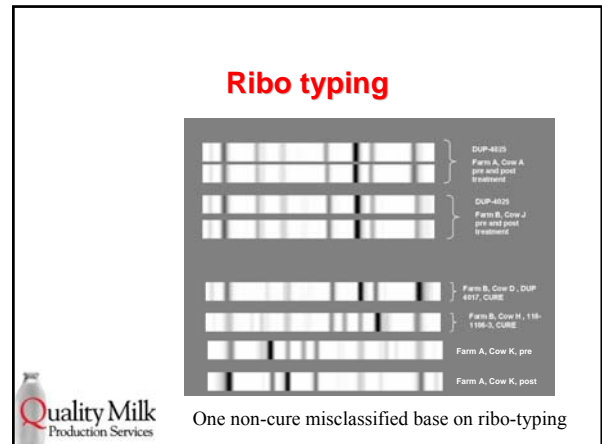
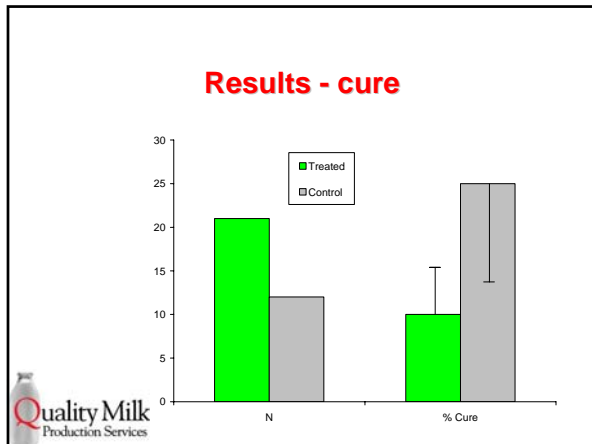
Day One:
5cc Immunoboost™ SC (under the skin)
30 cc BioCell® CBT SC
Washington Homeopathic “Staph” nosode 8 pills intravaginally/orally twice daily

Day Two
30 cc BioCell® CBT SC
Washington Homeopathic “Staph” nosode 8 pills intravaginally/orally twice daily

Day Three
30 cc BioCell® CBT SC
Washington Homeopathic “Staph” nosode 8 pills intravaginally/orally twice daily
Take composite sample for residue

Day Eight, Nine, Ten
30 cc BioCell® CBT SC





- ### Controlled *S.aureus* study
- Adequate design.
 - Not blind, but objective outcome.
 - Power adequate to find large difference (10% vs 35%).
 - Conclusion: null hypothesis of 'treatment and do-nothing-control are equivalent' cannot be rejected.
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- ### Conclusions
- Alternative therapies are a fact of life.
 - On-Farm Evaluations of alternative Therapy is possible.
 - Design of study determines the strength of the conclusions.
 - Randomize trials are doable and much needed.
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