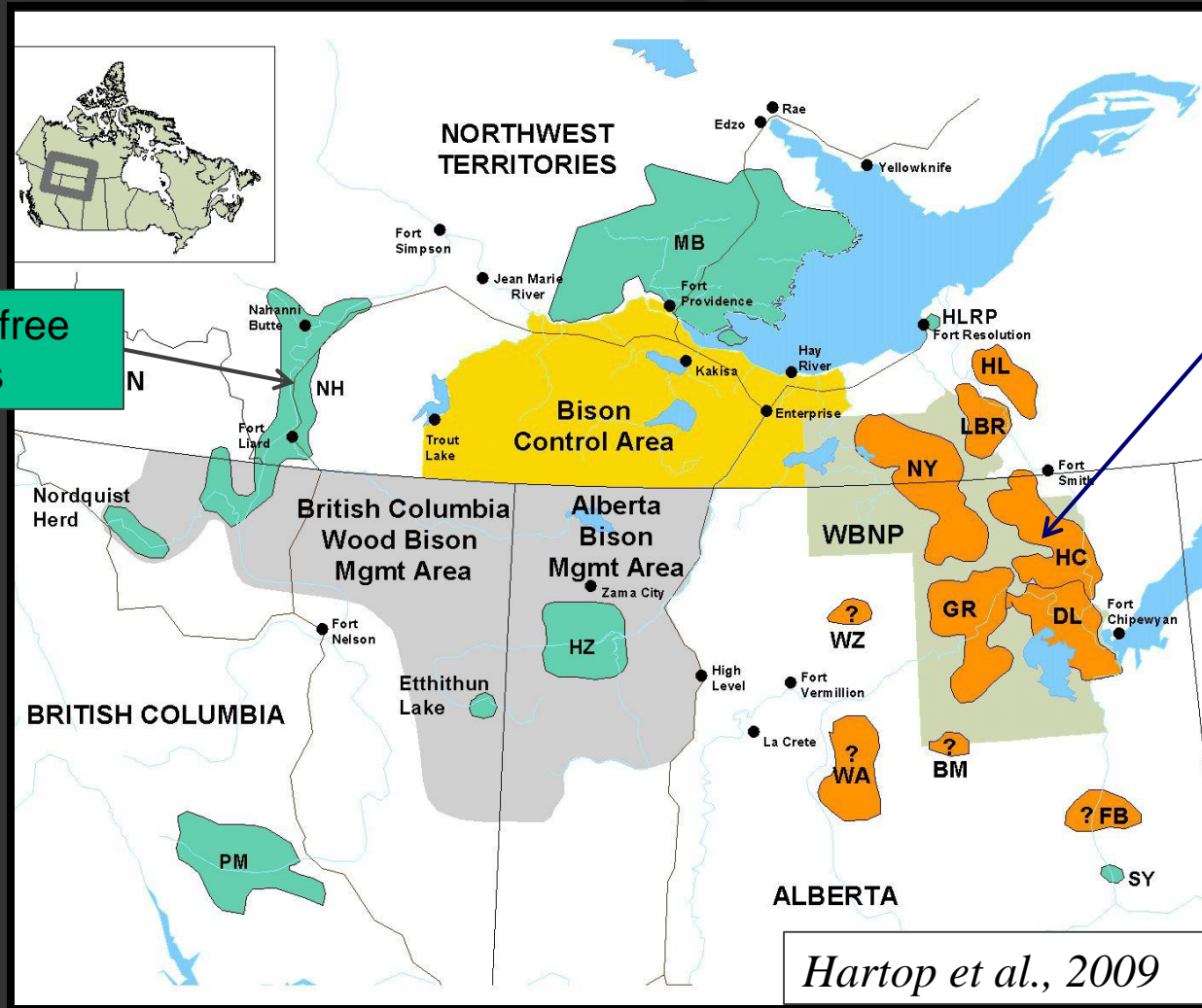


1st International Symposium on Bison Health

**Superovulation:
A tool to produce disease free
embryos in wood bison
(*Bison bison athabasca*)**

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Background



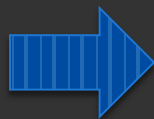
Genetic diversity

Brucellosis and tuberculosis

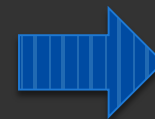
↓
Depopulation or control of infected bison population

↓
Loss of genetic diversity

Reproductive Technologies



Germ plasm bio-bank

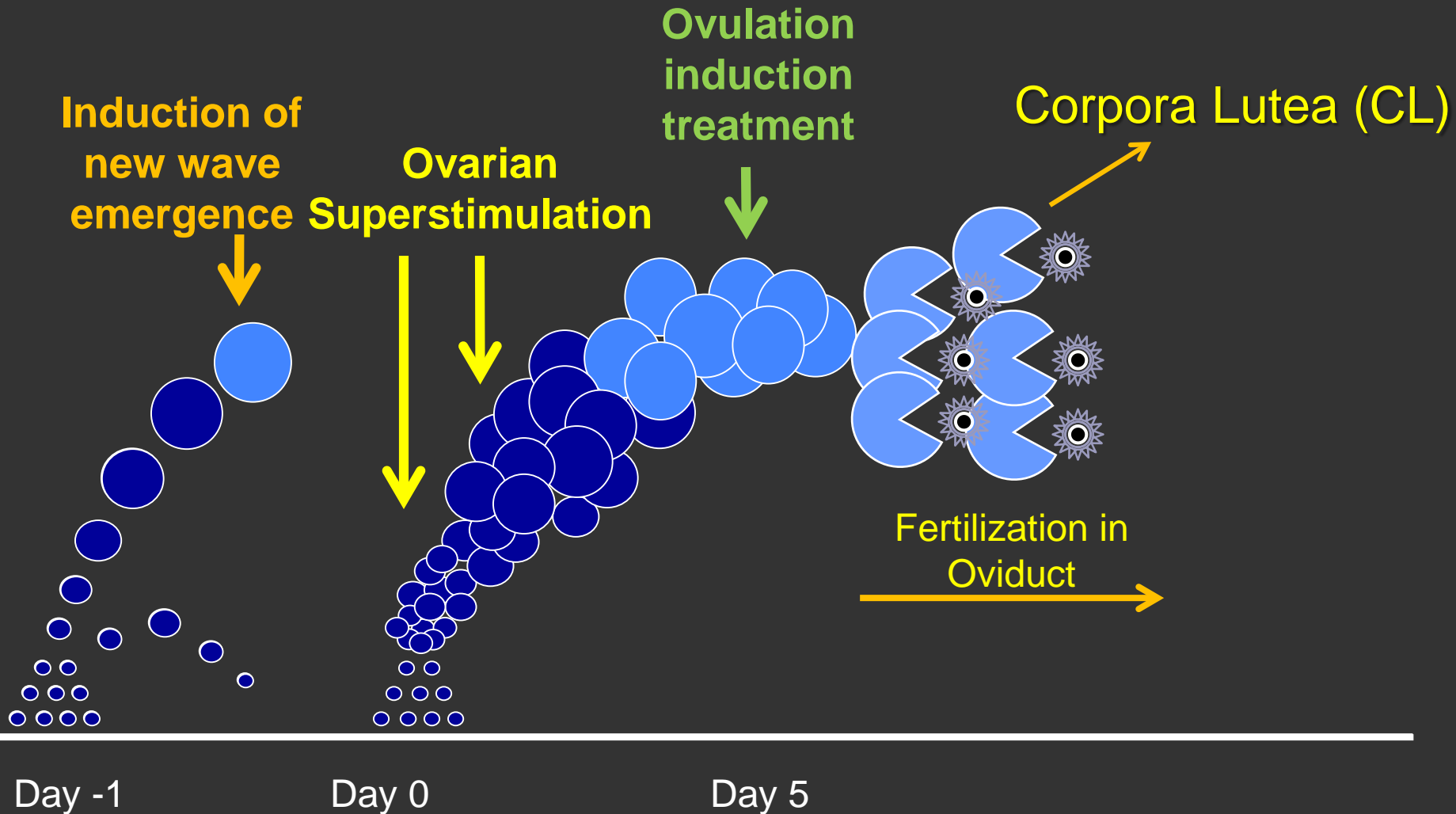


Disease-free offspring

Reproductive technologies to produce disease-free wood bison embryos

- In vitro embryo production
 - Superstimulation and oocyte collection
- In-vivo-derived embryos
 - Superovulation and embryo collection

Superovulation



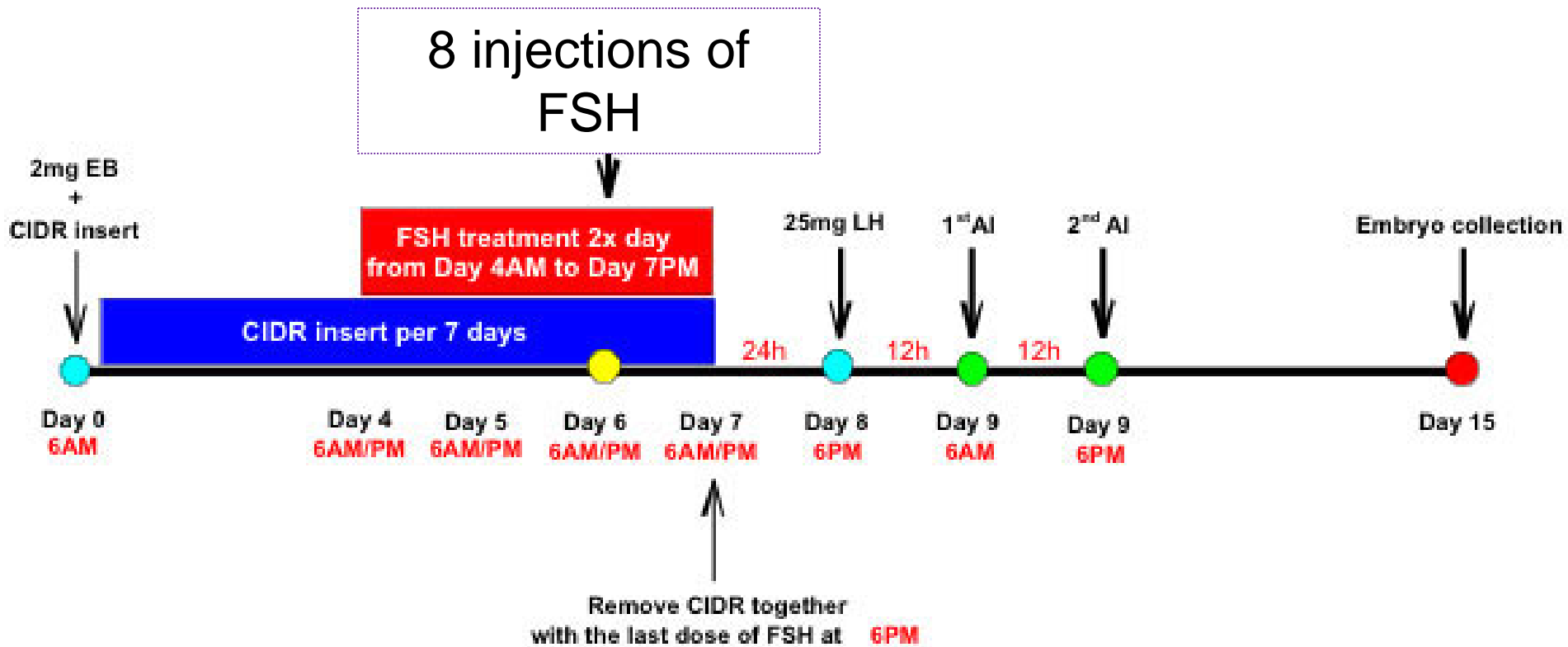
History of superovulation in bison

- Dorn et al., 1990
 - 3.1 CL & 0.4 embryos (n = 14)
- Robison et al., 1998
 - 1.9 CL & 0.6 embryos (n = 16)
- Othen et al., 1999
 - 1.0 CL (n = 10)



Stress of handling?

Superovulation and embryo collection: The bovine model



Superovulation in wood bison at the University of Saskatchewan

Palomino et al., 2012:

- Ovarian superstimulation with **two doses of FSH** diluted in 0.5% hyaluronan
- Induction of multiple ovulation with **hCG**

Toosi et al., 2013:

- Embryo transfer with fresh embryos
- First live wood bison ET calves



Animals and facilities

- Native Hoofstock Centre, University of Saskatchewan
- 50 adult female and 4 male wood bison



Hydraulic chute to handle bison

Synchronization: Follicular ablation



Day -1



Day 0

Superovulation



FSH 400 mg (Folltropin, Bioniche)



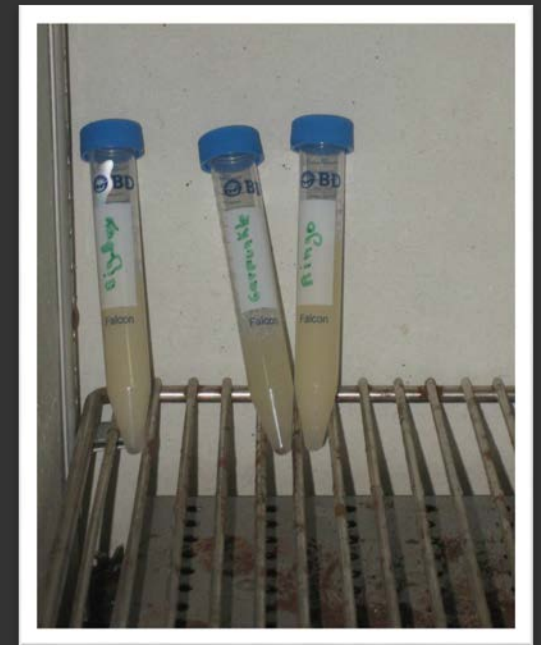
Hyaluronic acid (Map 5, Bioniche)



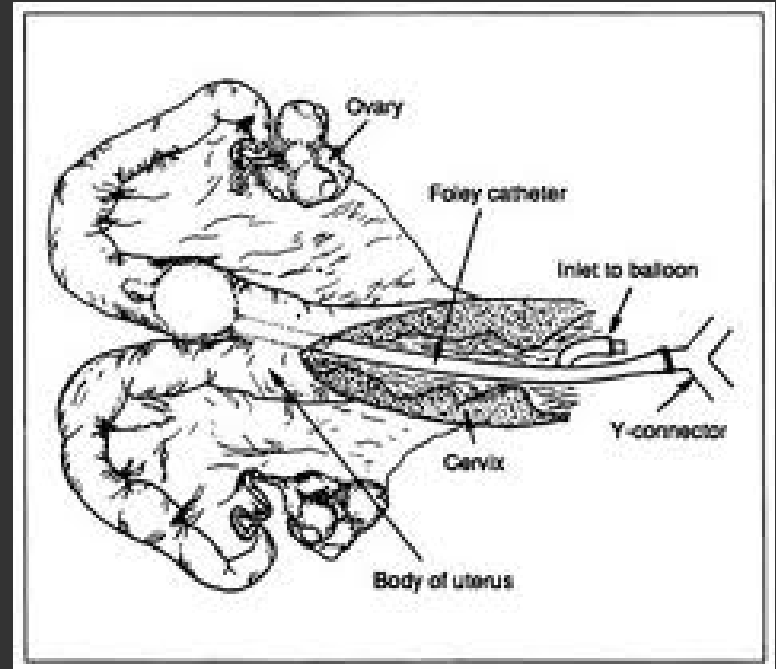
hCG 3000 IU (Chorulon, Merck)

Artificial Insemination: Chilled semen

- Semen collection: electro-ejaculation, 3 bulls, pooled
- Extender: Tryladil + 20% egg yolk
- Chilled semen 5°C (1-3 days)



Embryo collection: Non-surgical



Embryo classification (IETS, 2010)

QUALITY OF EMBRYOS²

Code 1	Excellent or Good
Code 2	Fair
Code 3	Poor
Code 4	Dead or Degenerating



Palomino et al., 2013 - 2014:

Superovulation & embryo collection throughout the year

Table 1. Superovulation and embryo collection in wood bison using simplified superovulatory protocols

	Non breeding season (Anovulatory)	Breeding season (Ovulatory)	P-Value
# Animals	48	44	
# Corpora lutea	7.2 ± 0.7	5.9 ± 0.5	0.15
# Ova/ embryos	3.7 ± 0.6	3.5 ± 0.4	0.78
# Embryos	2.9 ± 0.5	3.1 ± 0.3	0.69
# Grade 1 & 2 embryos	0.8 ± 0.2	2.0 ± 0.3	0.001

Palomino et al., 2014: (Ovulatory season)

Table 2. Effect of lengthening of FSH treatment (2 vs 3 doses) on superovulation and embryo quality in wood bison

	FSH x 2	FSH x 3	P - value
Number of bison	14	14	
Number of follicles ≥ 9 mm on Day 5	8.1 \pm 1.1	9.8 \pm 1.4	0.40
Ovulation rate (# ovulations / # follicles ≥ 9 mm)	60% (67/130)	74% (73/98)	0.07
Number of CL	4.6 \pm 0.6	6.5 \pm 0.9	0.13
Total ova-embryos	2.4 \pm 0.5	4.8 \pm 0.7	0.04
Grade 1 & 2	1.5 \pm 0.4	2.7 \pm 0.6	0.06
Grade 3	0.2 \pm 0.1	0.7 \pm 0.4	0.03
Grade 4	0.8 \pm 0.2	1.6 \pm 0.6	0.05

Summary

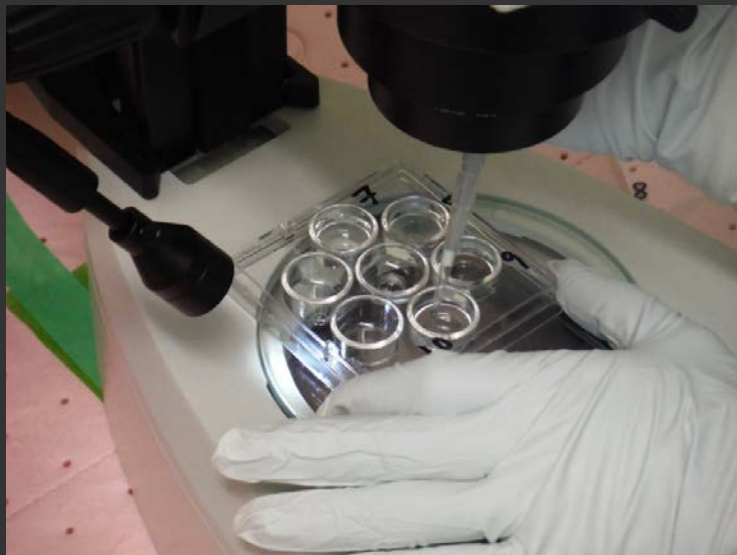
- Grade 1 & 2 embryos: 0.5 – 2
- Effect of season
 - Quality of embryos affected by season
- Greater quality of embryos: 3 doses of FSH?
- Next step:
 - Disease-free embryo production

Production of disease-free embryos from superovulated wood bison

Objective:

- Determine the effectiveness of washing procedures for removing *Brucella abortus* from wood bison embryos exposed *in vitro*

Level 3 laboratory – Intervac/VIDO



IETS Washing
procedures:
10 washes

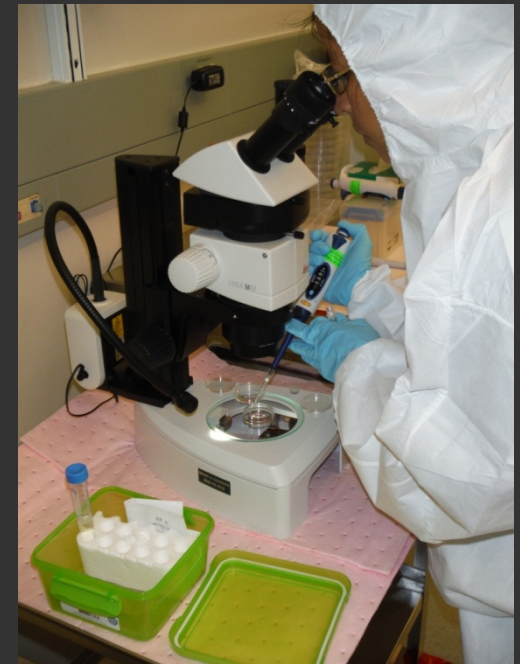


Table 3. Results of washing procedures to remove brucella bacteria from in vitro infected wood bison embryos

Replicate	Negative control	Positive control	Wash 1	Wash 3	Wash 6	Wash 9	# washed embryos	# of culture-positive embryos
<u>Without antibiotics</u>								
1	Neg	Pos	Pos	Neg	Neg	Neg	2	0
2	Neg	Pos	Pos	Neg	Neg	Neg	4	0
3	Neg	Pos	Pos	Neg	Neg	Neg	4	0
4	Neg	Pos	Pos	Neg	Neg	Neg	4	1
5	Neg	Pos	Pos	Pos	Neg	Neg	6	1
6	Neg	Pos	Pos	Pos	Neg	Neg	7	0
Total							27	2
<u>With antibiotics</u>								
1	Neg	Pos	Pos	Neg	Neg	Neg	2	0
2	Neg	Pos	Pos	Pos	Neg	Neg	4	0
3	Neg	Pos	Pos	Neg	Neg	Neg	4	0
4	Neg	Pos	Pos	Neg	Neg	Neg	4	0
5	Neg	Pos	Pos	Pos	Neg	Neg	6	0
6	Neg	Pos	Pos	Pos	Neg	Neg	7	0
Total							27	0

Conclusion: disinfection technique is effective

Acknowledgments

- Dr. Adams
- Dr. Mapletoft
- Dr. Woodbury
- Dr. McCorkell
- Dr. Anzar
- Dr. Mastromonaco
- Dr. Cervantes
- Reproduction lab colleagues



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