Appendix A2

Table of ICE Protocols

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Test Method Component	Prinsen (2005)	Prinsen (2000)	Prinsen (1996)	Balls et al. (1995) (INVITTOX Protocol)	Prinsen and Koeter (1993)
Time lapse from abattoir to test	≤ 2hr	≤ 2hr	≤ 2hr	≤ 2hr	≤ 2hr
Age of chicken	7 weeks	7 weeks	7 weeks	not specified	7 weeks
Weight of chicken	2.5-3.0 kg	2.5-3.0 kg	2.5-3.0 kg	not specified	2.5-3.0 kg
Number of eyes/ test material	3	3	5 or 3	at least 3	5
Corneal thickness deviation tolerance	>10% excluded	>10% excluded	>10% excluded	>10% excluded	>10% excluded
Length of equilibration period	45-60 min	45-60 min	45-60 min	45-60 min	45-60 min
Positive control	not included	not included	not included	not included	not included
Negative control	isotonic saline	isotonic saline	isotonic saline	isotonic saline	isotonic saline
Quantity applied	30 mL/30 mg	30 mL/30 mg	30 mL/30 mg	30 mL/30 mg	30 mL/30 mg
Exposure period	10 sec	10 sec	10 sec	10 sec	10 sec
Incubation temp	32±1.5°C	32±1.5°C	32±1.5°C	32±1.5°C	32±1.5°C
Perfusion rate	0.10-0.15 mL/min	0.10-0.15 mL/min	0.10-0.15 mL/min	0.10-0.15 mL/min	0.10-0.15 mL/min
Volume of rinse	20 mL saline	20 mL saline	20 mL saline	20 mL saline	20 mL saline
Slit lamp scope used	Haag-Streit scope	Haag-Streit scope	Haag-Streit scope	Haag-Streit scope	Haag-Streit scope
Evaluation times	0, 30, 75, 120, 180, 240 min PD	0, 30, 75, 120, 180, 240 min PD	0, 30, 75, 120, 180, 240 min PD	0, 30, 75, 120, 180, 240 min PD	0, 30, 75, 120, 180, 240 min PD

Test Method Component	Prinsen (2005)	Prinsen (2000)	Prinsen (1996)	Balls et al. (1995) (INVITTOX Protocol)	Prinsen and Koeter (1993)
Fluorescein observation time (2.0%)	30 min PD	30 min PD	30 min PD	30 min PD	30 min PD
Subjectivity of fluoroscein evaluation	0-3 based on #cells retaining fluoroscein	0-3 based on #cells retaining fluoroscein	0-3 based on #cells retaining fluoroscein	0-3 based on #cells retaining fluoroscein	0-3 based on #cells retaining fluoroscein
Subjectivity of corneal opacity	0-4 based on extent of opacification	0-4 based on extent of opacification	0-4 based on extent of opacification	0-4 based on extent of opacification	0-4 based on extent of opacification
Subjectivity of morphological findings	interpretation of investigator	interpretation of investigator	interpretation of investigator	interpretation of investigator	interpretation of investigator

[1]PD = Post dosing