

Veterinary Parasitology and Parasitic Diseases Department of Pathology and Animal Health Faculty of Veterinary Medicine, University of Naples Federico II Via della Veterinaria, 1 - 80137 Naples, Italy www.flotac.unina.it - www.parassitologia.unina.it

PREFACE

The FLOTAC Manual is divided into two parts:

The first part describes (a) basic principles; (b) components; (c) accessories; (d) assembly; and (e) positions and steps of the FLOTAC[®].

The second part of the Manual describes the Flotac techniques, i.e., the Flotac basic technique, the Flotac dual technique, the Flotac double technique, and the Flotac faecal egg count calibration.

Flotation solutions (FS) play a fundamental role in determining the sensitivity, precision and accuracy of any copromicroscopic technique (qualitative and/or quantitative) based upon flotation. The key role of FS is further discussed in the Flotac faecal egg count calibration section of the second part of the Manual.

Flotac techniques augment the efficiency of the various **FS** with respect to clarity of reading, sensitivity, flotation of numbers of parasitic elements, precision and accuracy; but they also augment the negative aspects of some **FS** regarding turbidity of reading, floating of small and large faecal debris, etc. As a consequence, not all the **FS** used in parasitological labs can be used with the Flotac techniques.

The section Flotation solutions of the Manual and of this <u>APPENDIX</u> reports the chemical composition of the 9 FS that give the best results using the Flotac techniques with respect to the clarity of reading, sensitivity, precision and accuracy.

The **WORK TABLES** in this **APPENDIX** report the classification of 9 FS for the most common parasitic elements eliminated with faeces from cattle, buffaloes, sheep and goats.

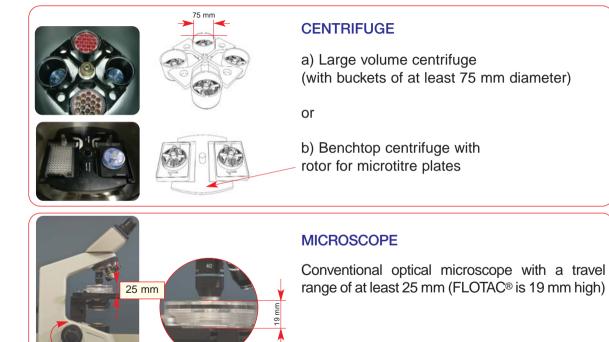
The Flotac techniques are designed for use by researchers, and all laboratory technicians who need highly accurate and precise results, where such results are more important than the simplicity or cost of the technique chosen.

It is our fond hope that the use of the Flotac techniques will help the advancement of knowledge in the fields of human and veterinary parasitology.

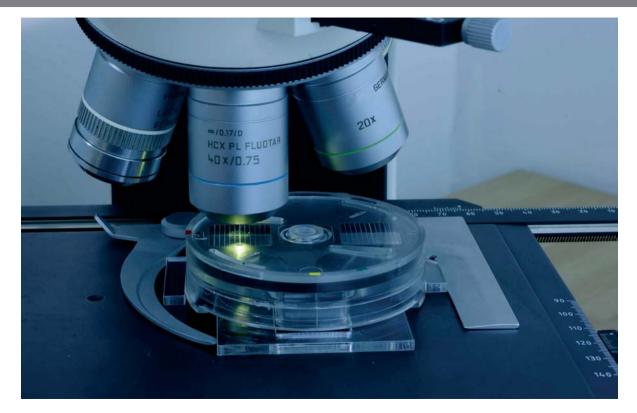
Prof. Giuseppe Cringoli

FLOTAC®

LABORATORY EQUIPMENT REQUIRED FOR THE FLOTAC TECHNIQUES



FLOTAC®



Flotation Solutions

Flotation solutions play a key role in determining the sensitivity, precision and accuracy of any copromicroscopic technique (qualitative and/or quantitative) based upon flotation.

Among the 14 flotation solutions listed in the paper by Cringoli et al. (Vet Parasitol 2004, 123: 121- 131), the following 9 flotation solutions give the best results with the Flotac techniques with respect to clarity of reading, sensitivity, precision and accuracy.

	Flotation solutions	Specific gravity (s.g.)
FS 1	Sheather's Sugar Solution	1.200
FS 2	Satured Sodium Chloride	1.200
FS 3	Zinc Sulphate 1.200	1.200
FS 4	Sodium Nitrate	1.200
FS 5	Sucrose and Potassium Iodomercurate (Rinaldi)	1.250
FS 6	Magnesium Sulphate	1.280
FS 7	Zinc Sulphate 1.350	1.350
FS 8	Potassium Iodomercurate	1.440
FS 9	Zinc Sulphate and Potassium Iodomercurate (Tampieri - Restani)	1.450

FS 1 - Sheather's Sugar Solution (s.g. - 1.200)

- 1 Combine 355 ml of water and 454 grams of granulated sugar (sucrose). Corn syrup and dextrose are not suitable substitutes.
- 2 Dissolve the sugar in the water by stirring on a magnetic stirrer over low or indirect heat (e.g., the top half of a double boiler). If the container is placed on a high direct heat source, the sugar may caramelize instead of dissolving in the water.
- 3 After the sugar is dissolved and the solution has cooled to room temperature, add 6 ml of formaldehyde (40%) USP to prevent microbial growth.
- 4 Check the s.g. with a hydrometer.

FS 2 - Satured Sodium Chloride (NaCl, s.g. - 1.200)

- 1 Combine 1000 ml of warm water and about 500 grams of salt until no more salt goes into solution and the excess settles on the bottom of the container.
- 2 Dissolve the salt in the water by stirring on a magnetic stirrer.
- 3 To ensure that the solution is fully saturated, it should be allowed to stand overnight at room temperature. If the remaining salt crystals dissolve overnight, more can be added to ensure that the solution is saturated.
- 4 Check the s.g. with a hydrometer, recognizing that the s.g. of saturated solution will vary slightly with environmental temperature.

FS 3 – Zinc Sulphate (ZnSO4-7H2O, s.g. - 1.200)

- 1 Combine 500 ml of water and 330 grams of zinc sulphate.
- 2 Dissolve the zinc sulphate in the water by stirring on a magnetic stirrer.
- 3 Add water to reach a final volume of 1000 ml.
- 4 Check the s.g. with a hydrometer.

FS 4 - Sodium Nitrate (NaNO3, s.g. - 1.200)

- 1 Combine 500 ml of water and 315 grams of sodium nitrate.
- 2 Dissolve the sodium nitrate in the water by stirring on a magnetic stirrer.
- 3 Add water to reach a final volume of 1000 ml.
- 4 Check the s.g. with a hydrometer.

FS 5 – Sucrose and Potassium Iodomercurate (Rinaldi) (s.g. 1.250)

- 1 Combine 600 ml of water and 600 grams of sucrose.
- 2 Dissolve the sugar in the water by stirring on a magnetic stirrer over low or indirect heat (e.g., the top half of a double boiler). If the container is placed on a high direct heat source, the sugar may caramelize instead of dissolving in the water.
- 3 After the sugar is dissolved and the solution has cooled to room temperature, add 20 ml of solution B (see below).
- 4 Check the s.g. with a hydrometer.

Solution B

- 1 Combine 100 grams of mercure iodide and 63 ml of water.
- 2 Stir vigorously.
- 3 Add 78 grams of potassium iodide and stir again.

FS 6 - Magnesium Sulphate (MgSO4, s.g. - 1.280)

- 1 Combine 500 ml of water and 350 grams of magnesium sulphate.
- 2 Dissolve the magnesium sulphate in the water by stirring on a magnetic stirrer.
- 3 Add water to reach a final volume of 1000 ml.
- 4 Check the s.g. with a hydrometer.

FS 7 - Zinc Sulphate (ZnSO4-7H2O, s.g. - 1.350)

- 1 Combine 685 ml of water and 685 grams of zinc sulphate.
- 2 Dissolve the zinc sulphate in the water by stirring on a magnetic stirrer.
- 3 Check the s.g. with a hydrometer.

FS 8 – Potassium Iodomercurate (s.g. - 1.440)

- 1 Combine 399 ml of water and 150 grams of mercure iodide.
- 2 Stir vigorously.
- 3 Add 111 grams of potassium iodide and stir again.
- 4 Check the s.g. with a hydrometer.

FS 9 - Zinc Sulphate and Potassium Iodomercurate (Tampieri - Restani) (s.g. - 1.450)

- 1 Combine 600 ml of water and 600 grams of zinc sulphate (ZnSO4-7H2O).
- 2 Dissolve the zinc sulphate in the water by stirring on a magnetic stirrer.
- 3 After the zinc sulphate is dissolved add the solution B (see below).
- 4 Check the s.g. with a hydrometer.

Solution B

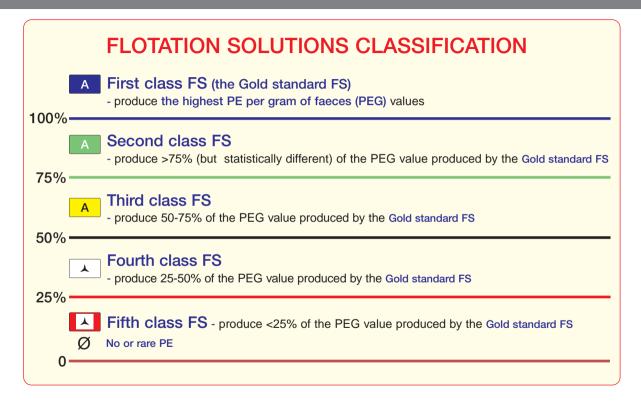
- 1 Combine 100 grams of mercure iodide and 63 ml of water.
- 2 Stir vigorously.
- 3 Add 78 grams of potassium iodide and stir again.

Flotac faecal egg count calibration and flotation solutions cassification

In the **WORK TABLES** reported in this **APPENDIX** for the parasitic elements (PE = eggs, larvae, oocysts, cysts) most frequently found in faeces from sheep, goats, cattle, and buffaloes, 9 flotation solutions (FS) divided into different classes (based on their efficiency) are reported.

The contents of the following WORK TABLES are the results of a series of Flotac faecal egg count calibrations (see Flotac Manual pg. 67) performed per each PE, utilizing composite faecal samples (both from grazing and housing animals), and utilizing four different methods of faecal preservation: fresh faeces, fixed in formalin 5%, fixed in formalin 10%, and frozen (see the scheme on page 17).

The classification of these results with respect to the efficiency of FS take into consideration both the 4 faecal preservation methods and the 9 FS within each faecal preservation method.



Coefficent of variation

Moreover, for each FS that produce PEG values above 50% of the value produced by the Gold standard FS, a letter indicates the respective coefficient of variation [CV = (standard deviation/PEG mean value resulting from 6 replicates) x 100] that represents the precision of the technique:

- (E) CV above 20%
- (D) CV between 15% and 20%
- (C) CV between 10% and 15%
- (B) CV between 5% and 10%
- (A) CV below 5%

The lower the CV is, the more precise is the technique; CV values below 5% are advisable.

The FS that produce PEG value below 50% of the value produced by the Gold standard FS are marked with (\checkmark). In addition the FS that produce PEG value of 0 (or rare PE) are marked with (Ø)

Each laboratory should have at least two FS for each PE of interest: a first choice FS and a second choice FS. The first choice - the Gold standard - FS are particularly recommended for research or diagnosis using the Flotac basic technique or the Flotac double technique when the faecal samples contain PE from a single parasitic species (natural or experimental mono-infection), or when the faecal samples contain different PE having the same behaviour with respect to the FS used.

The second and the third classes FS, are particularly useful for diagnosis used in parallel with the Gold standard FS and/or other classes FS on the same faecal sample with the Flotac dual technique in order to perform a wide parasitological screening on different PE. If necessary, also the subsequent classes of FS may be used.

FLOTATION SOLUTIONS CLASSIFICATION

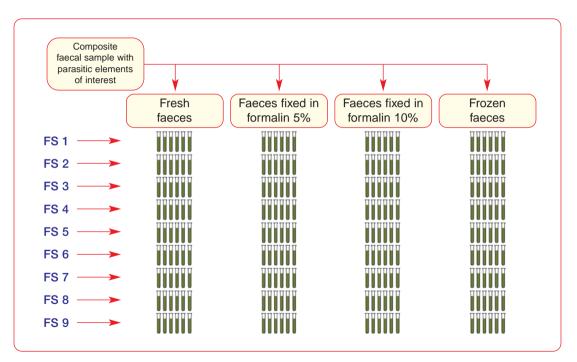
In diagnostic practice, a wide range of herbivorous alimentation types (which can produce undesirable residues in the faeces) may influence the clarity of reading with some of the 9 FS. At step n. 5 of the Flotac techniques (i.e., transfer into a tube), it is advisable to prepare an extra pellet. In case the FS used has a low clarity of reading due to the flotation of a large amount of debris, the technique can be repeated utilizing the other pellet with another compatible FS.

The following section contains:

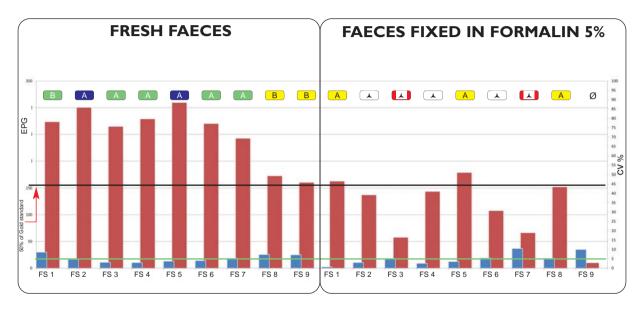
- a) The Flotac faecal egg count calibration (FFECC) scheme which was used for each parasitic element reported in the WORK TABLES (pg. 17).
- b) Four bar charts which show the results of the FFECC referred to GI strongyles in sheep for each faecal preservation method: an example of FS CLASSIFICATION. For each FS, the symbols corresponding to the classes of FS in the WORK TABLES are also reported above each chart (pgs 18 19).
- c) A planning which shows the overall results of the FFECC for GI strongyles in sheep: an example of FS CLASSIFICATION (pg. 20).
- d) The **WORK TABLES** which show, for each faecal preservation method, the results of the FFECC performed for the most common parasitic elements eliminated with the faeces from herbivores (pgs 22 31).

FLOTATION SOLUTIONS CLASSIFICATION

Flotac faecal egg count calibration scheme



Bar plot of results of Flotac faecael egg counts calibration of sheep

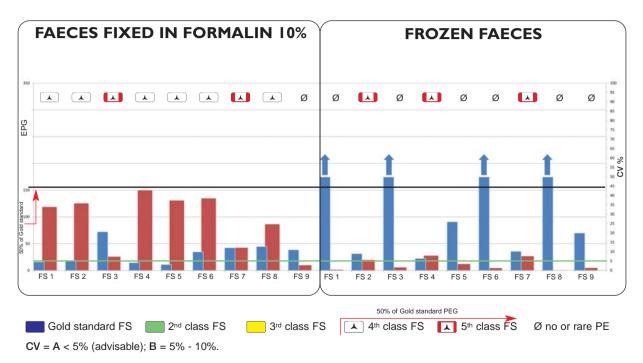


Mean number (derived from 6 replicates) of eggs per gram of faeces

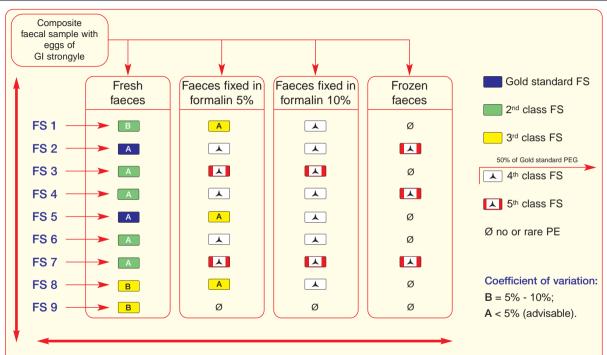
Coefficient of Variation (CV) = (standard deviation / mean PEG) x 100

50% of Gold standard PEG
CV below 5% (optimal)

gastrointestinal nematode: an example of FS CLASSIFICATION



Sheep - Flotac faecal egg count calibration on Gastrointestinal strongyle eggs



The classification of these results with respect to the efficiency of FS take into consideration both the 4 faecal preservation methods and the 9 FS within each faecal preservation method.

sheep and goats **WORK TABLES**

WORK TABLE

SHEEP AND GOATS

FRESH FAECES

			Flotation solutions (FS) of choice and specific gravity									
Parasitio	c elements	FS 1	FS 2	FS 3	FS 4	FS 5	FS 6	FS 7	FS 8	FS 9		
		1.200	1.200	1.200	1.200	1.250	1.280	1.350	1.440	1.450		
Oocysts	Eimeria					В	В			Ø		
	GI strongyles	В	А	Α	Α	Α	Α	Α	В	В		
	Moniezia	В	Α	Α	В	В	Α	В	Α	С		
Eggs	Fasciola	Ø	Ø	Ø	Ø	Ø	Ø	Α		В		
	Paramphistomum	Ø	Ø	Ø	Ø	Ø	Ø	С	С	С		
	Dicrocoelium	Ø	Ø	Ø	Ø	Ø	Ø	Α	Α	Α		
Larvae (L1)	Lungworms	Ø	Ø	Α	Ø	Ø		В		Α		

Gold standard FS
2 nd class FS
3 rd class FS
🔺 4 th class FS
▲ 5 th class FS
Ø no or rare parasitic elements

Coefficient of variation

 $\begin{array}{l} D=15\% - 20\%;\\ C=10\% - 15\%;\\ B=5\% - 10\%;\\ A<5\% \mbox{ (advisable)}. \end{array}$

FRESH FAECES

1 st combination suggested							
FS 5	FS 9						
Eimeria GI strongyles Moniezia	Fasciola Paramphistomum Dicrocoelium Muellerius Cystocaulus Protostrongylus Neostongylus Dictyocaulus						

2 nd combination suggested							
FS 2	FS 7						
Eimeria	Fasciola						
GI strongyles	Paramphistomum						
Moniezia	Dicrocoelium						
	Muellerius						
	Cystocaulus						
	Protostrongylus						
	Neostongylus						
	Dictyocaulus						

FAECES FIXED IN FORMALIN 5%

			Flotation solutions (FS) of choice and specific gravity									
Parasitio	c elements	FS 1	FS 2	FS 3	FS 4	FS 5	FS 6	FS 7	FS 8	FS 9		
		1.200	1.200	1.200	1.200	1.250	1.280	1.350	1.440	1.450		
Oocysts	Eimeria	D		Ø						Ø		
	GI strongyles	Α				Α			Α	Ø		
	Moniezia	В	Α	Α		Α	С	В	В	В		
Eggs	Fasciola	Ø	Ø	Ø	Ø	Ø	Ø	В		Α		
	Paramphistomum	Ø	Ø	Ø	Ø	Ø	Ø		E	Α		
	Dicrocoelium	Ø	Ø	Ø	Ø	Ø	Ø	Α	Α	Α		
Larvae (L1)	Lungworms	*	Ø	В	Ø			Α		C		



Coefficient of variation

 $\begin{array}{l} \textbf{E} > 20\% \\ \textbf{D} = 15\% - 20\%; \\ \textbf{C} = 10\% - 15\%; \\ \textbf{B} = 5\% - 10\%; \\ \textbf{A} < 5\% \mbox{ (advisable)}. \end{array}$

FAECES FIXED IN FORMALIN 5%

1 st combination suggested							
FS 1	FS 9						
Eimeria GI strongyles Moniezia	Fasciola Paramphistomum Dicrocoelium Muellerius Cystocaulus Protostrongylus Neostongylus Dictyocaulus						

2 nd combination suggested							
FS 1	FS 7						
Eimeria GI strongyles	Fasciola Paramphistomum						
Moniezia	Dicrocoelium						
	Muellerius Cystocaulus						
	Protostrongylus						
	Neostongylus						
	Dictyocaulus						

FAECES FIXED IN FORMALIN 10%

			Flotation solutions (FS) of choice and specific gravity								
Parasitio	c elements	FS 1	FS 2	FS 3	FS 4	FS 5	FS 6	FS 7	FS 8	FS 9	
		1.200	1.200	1.200	1.200	1.250	1.280	1.350	1.440	1.450	
Oocysts	Eimeria			Ø			Ø	Ø		Ø	
	GI strongyles									Ø	
	Moniezia	В	Α	В	В	Α	В	В	С	В	
Eggs	Fasciola	Ø	Ø	Ø	Ø	Ø	Ø			E	
	Paramphistomum	Ø	Ø	Ø	Ø	Ø	Ø			E	
	Dicrocoelium	Ø	Ø	Ø	Ø	Ø			В		
Larvae (L1)	Lungworms		Ø		Ø	Ø			Ø	В	

Gold standard FS
2 nd class FS
3 rd class FS
▲ 4 th class FS
▲ 5 th class FS
Ø no or rare parasitic elements

Coefficient of variation

 $\begin{array}{l} {\sf E} > 20\% \\ {\sf D} = 15\% - 20\%; \\ {\sf C} = 10\% - 15\%; \\ {\sf B} = 5\% - 10\%; \\ {\sf A} < 5\% \mbox{ (advisable)}. \end{array}$

FAECES FIXED IN FORMALIN 10%

1 st combination suggested							
FS 5	FS 9						
Eimeria	Fasciola						
GI strongyles	Paramphistomum						
Moniezia	Dicrocoelium						
	Muellerius						
	Cystocaulus						
	Protostrongylus						
	Neostongylus						
	Dictyocaulus						

2 nd combination suggested							
FS 2	FS 7						
Eimeria	Fasciola						
GI strongyles	Paramphistomum						
Moniezia	Dicrocoelium						
	Muellerius						
	Cystocaulus						
	Protostrongylus						
	Neostongylus						
	Dictyocaulus						

FROZEN FAECES

		Flotation solutions (FS) of choice and specific gravity									
Parasitio	Parasitic elements		FS 2	FS 3	FS 4	FS 5	FS 6	FS 7	FS 8	FS 9	
		1.200	1.200	1.200	1.200	1.250	1.280	1.350	1.440	1.450	
Oocysts	Eimeria	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	
	GI strongyles	Ø		Ø		Ø	Ø		Ø	Ø	
	Moniezia	В	В	В	В	В	В	В	В	Α	
Eggs	Fasciola	Ø	Ø	Ø	Ø	Ø	Ø			Α	
	Paramphistomum	Ø	Ø	Ø	Ø	Ø	Ø		С	В	
	Dicrocoelium	Ø	Ø	Ø	Ø	Ø			Α	Α	
Larvae (L1)	Lungworms	Ø	Ø	В	Ø	Ø				В	



Coefficient of variation

 $\begin{array}{l} D = 15\% - 20\%;\\ C = 10\% - 15\%;\\ B = 5\% - 10\%;\\ A < 5\% \mbox{ (advisable)}. \end{array}$

FROZEN FAECES

1 st combination suggested								
FS 2	FS 9							
Eimeria	Moniezia							
GI strongyles	Fasciola							
	Paramphistomum							
	Dicrocoelium							
	Muellerius							
	Cystocaulus							
	Protostrongylus							
	Neostongylus							
	Dictyocaulus							

2 nd combination suggested								
FS 2	FS 7							
Eimeria	Fasciola							
GI strongyles	Paramphistomum							
Moniezia	Dicrocoelium							
	Muellerius							
	Cystocaulus							
	Protostrongylus							
	Neostongylus							
	Dictyocaulus							

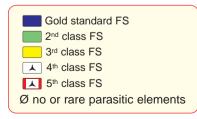
cattle and buffaloes **WORK TABLES**

WORK TABLE

CATTLE AND BUFFALOES

FRESH FAECES

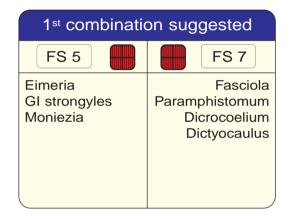
		Flotation solutions (FS) of choice and specific gravity									
Parasitio	Parasitic elements		FS 2	FS 3	FS 4	FS 5	FS 6	FS 7	FS 8	FS 9	
		1.200	1.200	1.200	1.200	1.250	1.280	1.350	1.440	1.450	
Oocysts	Eimeria		E		Ø	E	E			E	
	GI strongyles	В	E	С	E	C	E	E			
	Moniezia	D	E	E	E	E	E	E		D	
Eggs	Fasciola	Ø	Ø	Ø	Ø	Ø	Ø	Α		В	
	Paramphistomum	Ø	Ø	Ø	Ø	Ø	Ø	C	С	С	
	Dicrocoelium	Ø	Ø	Ø	Ø	Ø	Ø	A	Α	Α	
Larvae (L1)	Lungworms	Ø	Ø	Α	Ø	Ø		В		Α	



Coefficient of variation

 $\begin{array}{l} \textbf{E} > 20\% \\ \textbf{D} = 15\% - 20\%; \\ \textbf{C} = 10\% - 15\%; \\ \textbf{B} = 5\% - 10\%; \\ \textbf{A} < 5\% \mbox{ (advisable)}. \end{array}$

FRESH FAECES



2 nd combination suggested									
FS 1	FS 7								
Eimeria GI strongyles Moniezia	Fasciola Paramphistomum Dicrocoelium Dictyocaulus								

FAECES FIXED IN FORMALIN 5%

			Flotation solutions (FS) of choice and specific gravity									
Parasitio	Parasitic elements		FS 2	FS 3	FS 4	FS 5	FS 6	FS 7	FS 8	FS 9		
		1.200	1.200	1.200	1.200	1.250	1.280	1.350	1.440	1.450		
Oocysts	Eimeria			Ø	Ø		Ø	Ø				
	GI strongyles									Ø		
	Moniezia	E		E						D		
Eggs	Fasciola	Ø	Ø	Ø	Ø	Ø	Ø	В		Α		
	Paramphistomum	Ø	Ø	Ø	Ø	Ø	Ø		E	Α		
	Dicrocoelium	Ø	Ø	Ø	Ø	Ø	Ø	Α	Α	Α		
Larvae (L1)	Lungworms		Ø	В	Ø			Α		С		



Coefficient of variation

 $\begin{array}{l} {\sf E} > 20\% \\ {\sf D} = 15\% - 20\%; \\ {\sf C} = 10\% - 15\%; \\ {\sf B} = 5\% - 10\%; \\ {\sf A} < 5\% \mbox{ (advisable)}. \end{array}$

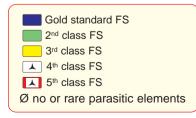
FAECES FIXED IN FORMALIN 5%

1 st combination suggested								
FS 5	FS 9							
Eimeria GI strongyles	Moniezia Fasciola Paramphistomum Dicrocoelium Dictyocaulus							

2 nd combination suggested								
FS 1	FS 7							
Eimeria GI strongyles	Moniezia Fasciola Paramphistomum Dicrocoelium Dictyocaulus							

FAECES FIXED IN FORMALIN 10%

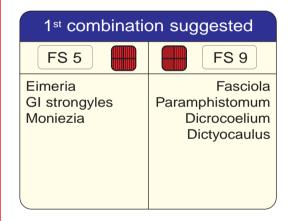
			Flotation solutions (FS) of choice and specific gravity									
Parasitio	Parasitic elements		FS 2	FS 3	FS 4	FS 5	FS 6	FS 7	FS 8	FS 9		
		1.200	1.200	1.200	1.200	1.250	1.280	1.350	1.440	1.450		
Oocysts	Eimeria		Ø	Ø	Ø			Ø		Ø		
	GI strongyles							Ø		Ø		
	Moniezia					D						
Eggs	Fasciola	Ø	Ø	Ø	Ø	Ø	Ø			E		
	Paramphistomum	Ø	Ø	Ø	Ø	Ø	Ø			E		
	Dicrocoelium	Ø	Ø	Ø	Ø	Ø			В			
Larvae (L1)	Lungworms		Ø		Ø	Ø			Ø	В		



Coefficient of variation

 $\begin{array}{l} {\sf E} > 20\% \\ {\sf D} = 15\% - 20\%; \\ {\sf C} = 10\% - 15\%; \\ {\sf B} = 5\% - 10\%; \\ {\sf A} < 5\% \mbox{ (advisable)}. \end{array}$

FAECES FIXED IN FORMALIN 10%



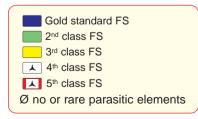
2 nd combination suggested								
FS 1	FS 7							
Eimeria GI strongyles Moniezia	Fasciola Paramphistomum Dicrocoelium Dictyocaulus							

WORK TABLE

CATTLE AND BUFFALOES

FROZEN FAECES

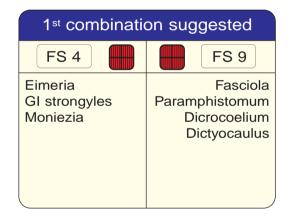
		Flotation solutions (FS) of choice and specific gravity									
Parasitio	Parasitic elements		FS 2	FS 3	FS 4	FS 5	FS 6	FS 7	FS 8	FS 9	
		1.200	1.200	1.200	1.200	1.250	1.280	1.350	1.440	1.450	
Oocysts	Eimeria									Ø	
	GI strongyles	Ø		Ø		Ø	Ø	Ø		Ø	
	Moniezia			E	E						
Eggs	Fasciola	Ø	Ø	Ø	Ø	Ø	Ø			Α	
	Paramphistomum	Ø	Ø	Ø	Ø	Ø	Ø		С	В	
	Dicrocoelium	Ø	Ø	Ø	Ø	Ø			Α	Α	
Larvae (L1)	Lungworms	Ø	Ø	В	Ø	Ø				В	



Coefficient of variation

 $\begin{array}{l} \mathsf{E} > 20\% \\ \mathsf{D} = 15\% - 20\%; \\ \mathsf{C} = 10\% - 15\%; \\ \mathsf{B} = 5\% - 10\%; \\ \mathsf{A} < 5\% \mbox{ (advisable)}. \end{array}$

FROZEN FAECES



2 nd combination suggested								
FS 4	FS 7							
Eimeria GI strongyles Moniezia	Fasciola Paramphistomum Dicrocoelium Dictyocaulus							