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CHROMagar™
The Chromogenic Media Pioneer

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Performance of CHROMagar™ Pasteurella

Chromogenic Culture Medium for the Detection of Pasteurellaceae

Laboratory

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This report contains 12 pages, including 1 page of annexes

Table of Contents

1. Introduction

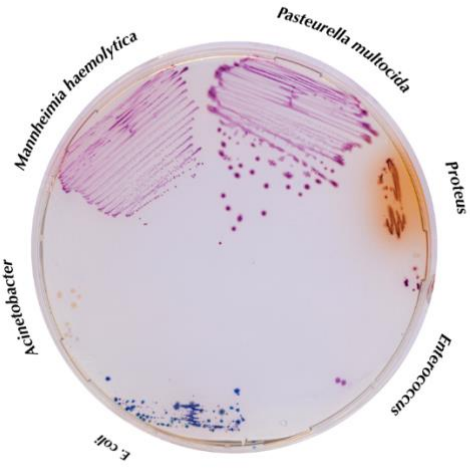
Bovine respiratory disease (BRD) affects young cattle every year during the cold, wet season throughout the world. Bovine pneumonic pasteurellosis is a respiratory disease that continuous to have a significant impact on livestock production, as infected animals can have reduced growth rates, increased mortality rates and produce less milk, resulting in financial losses for farmers (Wilson & Ho, 2013).

The infectious agents responsible for the primary respiratory lesions are viruses and pathogen bacteria such as *Mycoplasma bovis*. However, *Pasteurellaceae* bacteria commensal to upper respiratory tract of cattle, such as *Pasteurella multocida*, *Mannheimia haemolytica* and *Histophilus somni*, can then cause superinfections and more serious bronchopneumonia lesions. Moreover, BDR can spread rapidly within animal populations, especially in crowded or poorly managed environments (Wilson & Ho, 2013). *Pasteurellaceae* species have shown the ability to develop resistance to multiple antibiotics, which poses challenges in the treatment of infected animals (Klima *et al.*, 2014).

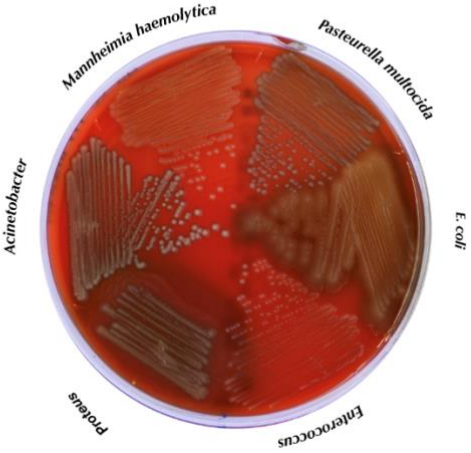
Early detection and diagnosis of *Pasteurellacea*e infections in animals are crucial for several reasons, including the administration of timely targeted antibiotic therapy and monitoring the emergence and spread of antibiotic resistance in order to implement effective disease control measures and minimise economic losses.

Unhealthy lung tissue specimens from ruminants are routinely tested for β-haemolysis of *M. haemolytica* on blood agar plates (Delano *et al*. 2002) where annex flora can interfere with the detection of the infectious agent. Although all the isolates of β-haemolytic *M. haemolytica* are considered to produce virulent leukotoxin, conflicting reports exist in literature. Indeed, the study of Bavananthasivam and co-workers (2018) showed that 55% of the isolates that did not produce leukotoxin protein, were haemolytic. Moreover, not all the isolates produced leukotoxin proteins, some isolates that produced leukotoxin were not leukotoxic and some not leukotoxic were haemolytic. Therefore, β-haemolysis may not be a reliable indicator of leukotoxicity of *M. haemolytica* isolates.

CHROMagar™ Pasteurella was developed to improve the detection of Pasteurellaceae from bovine respiratory samples (*e.g.*, nasal swab, trans-tracheal aspiration, and lung). This chromogenic medium, not based on haemolysis detection, allows the isolation and differentiation of *Pasteurellaceae* colonies in mauve coloration (see below) by the inhibition or blue counter-coloration of the annex flora after overnight aerobic incubation.



CHROMagar™ Pasteurella



Blood agar

In addition, respiratory tract samples or abscesses samples at the site of the bite or scratch from domestic animals such as cats and dogs can also be incubated on CHROMagar™ Pasteurella plates when *P. multocida* infections are suspected.

CHROMagar™ Pasteurella is composed of a powder base medium and two supplements. The powder base and supplement S1 are stored at 15 to 30 °C, the storage temperature of supplement S2 is 2 to 8 °C. Veterinary samples like nose swabs, broncho-alveolar lavage samples can be processed by direct streaking or spreading onto CHROMagar™ Pasteurella plates. Medium plates are incubated in aerobic atmosphere at 37 °C for 18 to 24 h. For detection of *H. somni*, plates are incubated in a 5% CO_2 atmosphere at 37 °C for 36 to 48 h.

This document compiles CHROMagar™ Pasteurella evaluations at two stages:

- In-house evaluations of the chromogenic formula with pure strains.
- Independent laboratory evaluations of the CHROMagar™ Pasteurella culture medium using respiratory tract samples and respiratory organ specimens.

2. In-house evaluation of the chromogenic formula

2.1. Inclusivity results

Pasteurella multocida and *Mannheimia haemolytica* strains were tested on CHROMagar™ Pasteurella plates, incubated in an O_2 atmosphere at 37°C for 24 h to assess the inclusivity of the chromogenic formula. Similarly, the inclusivity of *Histophilus somni* was assessed in a 5% CO_2 atmosphere at 37°C for 36-48 h. In these studies, Columbia agar plates with 5% sheep blood (BA) were used as the reference medium.

Table I. Bacterial strains tested to evaluate inclusivity of CHROMagar™ Pasteurella.

Bacterial Species	Strain #	CHROMagar™ Pasteurella
Pasteurella multocida	AR6371	M++, 0.5 - 2 mm
P. multocida	AR6372	M++, 1 - 3 mm
P. multocida	AR6373	M++, 1 - 3 mm
P. multocida	AR6374	M+/-, 0.6 - 1 mm
P. multocida	AR6375	M++, 0.5 - 2 mm
P. multocida	ATCC® 43137	M+, 2 - 3 mm
P. multocida subsp. *multocida*	ATCC® 12945	M++, 2 - 3 mm
P. aerogenes	AR6367	M+, 1 mm
Mannheimia haemolytica	AR6368	M++, 0.8 - 1 mm
M. haemolytica	AR6369	M++, 0.8 - 1 mm
M. haemolytica	AR6370	M++, 0.5 - 0.6 mm
M. haemolytica	ATCC® 33396	M+, 0.5 - 0.8 mm
Histophilus somni	ATCC® 43625	M+, 0.5 - 1.5 mm (48 h at 37°C in 5% CO_2)

M, mauve; +, - color intensity; size in mm; AR, CHROMagar™ strain collection.

The chromogenic formula showed 100% inclusivity with colony coloration in mauve, after a 24h aerobic incubation at 37°C for twelve strains of *Pasteurellaceae*. One strain of *H. somni* was found to be among the inclusive species growing mauve when the medium plates were incubated in a 5% CO_2 atmosphere at 37°C for 36 to 48 h.

2.2. Exclusivity results

The exclusivity of CHROMagar™ Pasteurella medium was assessed with several microbial strains after incubation in an O_2 atmosphere at 37 °C for 24 h. TSA plates were used as the reference medium.

Table II. Microbial strains tested to evaluate exclusivity of CHROMagar™ Pasteurella culture medium.

Microbial Species	Strain #	CHROMagar™ Pasteurella
Staphylococcus aureus subsp. *aureus*	ATCC® 25923	Small DZ, M
Staphylococcus saprophyticus	ATCC® BAA-750	-
Staphylococcus haemolyticus	AR5908	unc. small col. in DZ
Streptococcus bovis	AR5124	-
Streptococcus uberis	ATCC® 700407	µDZ
Streptococcus agalactiae	ATCC® 13813	-
Bacillus cereus	CIP 5832	-
Lactobacillus spp.	AR5215	-
Leuconostoc spp.	AR4341	-
Rothia mucilaginosa	AR5907	Small DZ, M--/+
Acinetobacter baumannii	AR5193	unc. DZ, 4 mm
A. baumannii	AR5610	unc., <20 colonies in DZ
A. baumannii	AR5620	unc./P, 3.5 mm
A. baumannii	AR5280	unc., 1.5 mm
Escherichia coli	ATCC® 25922	B, col. in DZ, 4 - 4.5 mm
E. coli	NCTC 13476	B/gray, 3 mm
Klebsiella aerogenes	ATCC® 13048	MB+, 4 mm
Klebsiella pneumoniae subsp. *pneumoniae*	ATCC® 13883	MB, col. in DZ
Klebsiella pneumoniae	ATCC® BAA-170	MB/violet, 3 mm
Enterobacter aerogenes	ATCC® 13048	col. in DZ
Enterococcus casseliflavus	ATCC® 700327	Small DZ V+
Proteus mirabilis	AR3022	unc., swarming
Proteus	AR5075	unc., 2 - 2.5 mm
Proteus vulgaris	AR3919	DZ; 0.8 - 1.5 mm swarming ++
Serratia marcescens	NCTC 10211	col. in DZ
Enterococcus faecalis	ATCC® 29212	Small DZ M++
Citrobacter freundii	ATCC® 8090	M, col. in DZ
Pseudomonas aeruginosa	ATCC® 10145	DZ, P+/-
P. aeruginosa	ATCC® 9027	unc. P+/-, 2 – 3 mm
Bordetella bronchiseptica	ATCC® 10580	unc. col. ++, < 1 mm
Candida tropicalis	ATCC® 1369	-
Mold	AR4585	-

M, mauve; MB, metallic blue; B, blue; P, pink; V, violet; unc., uncolored; +, - color/swarming intensity; DZ, dense zone; col., colonies; size in mm; -, growth absence; AR, CHROMagar™ strain collection.

The chromogenic formula showed 100 % exclusivity for thirty-two bacterial and fungal species which were either inhibited or showed a blue/metallic blue counter-coloration.

3. Independent laboratory data

3.1. Medium performance with bacterial strains

Culture medium plates were prepared in the laboratory using different batches of CHROMagar™ Pasteurella base and CHROMagar™ Pasteurella supplements. These pre-poured media were sent to different laboratories to compare their performance to routine culture media.

Table III. Bacterial strains to evaluate sensitivity and specificity of CHROMagar™ Pasteurella.

Laboratory, Country	# of strains	Sensitivity	Specificity	Comments
Oniris site de la Chantrerie, Nantes, France	*M. haemolytica* (n=7) *P. multocida* (n=5) *Pasteurella* spp. (n=1)	100 %	100 %	-
School of Veterinary Medicine University of Copenhagen Veterinary Clinical Microbiology, Copenhagen, Danemark	*M. haemolytica* (n=7) *Bibersteinia* (n=7) Non-*Pasteurellaceae* (n=1)	100 %	100 %	Different serovars of *M. haemolytica* were tested. Culture medium in powder.
Université de Liège, Faculté de Médecine vétérinaire, Liège, Belgium	*P. multocida* (n=17) *M. haemolytica* (n=6) *P. cabali* (n=1) *P. aerogenes* (n=1) *H. somni* (n=2) * Non-*Pasteurellaceae* (n=7) *Actinobacillus pleuropneumoniae* (n=3)	93 % (Uncolored *H. somni*)	100 %	Plates were Incubated 24h at 37°C in a 5% CO_2 atmosphere. * Incubated 48h at 37°C in anaerobic atmosphere.
Ghent University Laboratory of Veterinary Bacteriology and Mycology, Ghent, Belgium	*P. multocida* (n=1) *M. haemolytica* (n=2) *Bibersteinia* (n=2) *Gallibacterium* (n=2) *H. somni* (n=2) * Non-*Pasteurellaceae* (n=2)	70 % (Uncolored *H. somni*)	100%	* Incubated 48h at 37°C in 5 % CO_2 atmosphere.
LabMediaServis s.r.o., Jaroměř, Czech Republic	*P. multocida* (n=6) *P. dagmatis* (n=1)	100 %	100 %	-

Aerobic incubation, 24h at 37°C or as in comments.

Target bacteria species were detected in mauve on CHROMagar™ Pasteurella plates which showed equivalent fertility compared to blood agar.

3.2. Medium performance with field samples

3.2.1. Detection of *Pasteurellaceae* species in respiratory tract samples

Laboratories in different countries poured plates from dehydrated medium. The results of the comparison between CHROMagar™ Pasteurella and blood agar are shown below.

Table IV. Respiratory tract samples tested on CHROMagar™ Pasteurella.

Laboratory, Country	Type of sample (# of samples)	Sensitivity	Specificity	Colony identification	Comments
Ghent University Laboratory of Veterinary Bacteriology and Mycology, Ghent, Belgium	Bovine nasal swabs and BAL (n=30)	90,5 % *P. multocida* (n=11) *M. haemolytica* (n=4) M. varigena (n=3) *H. somni* (n=1) *	ND	Maldi tof	* Incubated 48h at 37°C in an 5 % CO_2 atmosphere.
Arsia asbl., Ciney, Belgium	Bovine nasal swabs and BAL (n=11)	100 % *P. multocida* (n=4)	100 % *E. coli* colonies are colored in bleu	Maldi tof	Blood agar showed 80 % sensibility.
Vetdiagnostix, Midrand, South Afrika	Bovine respiratory samples (n=27)	100 % *P. multocida* (n=17) *M. haemolytica* (n=5) *H. somni* (n=1) *	100 %	ND	Medium in powder * Incubated 48h at 37°C.
Harran University Siirt University Faculties of Veterinary Medicine, Şanlıurfa and Siirt, Turkey	Bovine nasal swabs (n=84)	92.9 % *P. multocida* (n=26) 100 % *M. haemolytica* (n=2)	ND	PCR	Tel *et al*., 2022. *Vet. Sci. Pract.* **17:** 81-86.

ND, not determined; BAL, broncho-alveolar lavages.

CHROMagar™ Pasteurella can be used to monitor the presence of *Pasteurellaceae* in respiratory tract samples from cattle. *M. haemolytica* generally develops in smaller colonies than *P. multocida*.

Some veterinary samples from Western College of Veterinary Medicine in the University of Saskatchewan, Canada were reported to contain mauve colonies which were identified as *E. coli*. This appears to be a rare case considering the biochemical features of this bacterium.

CHROMagar™ Pasteurella plates were also employed by end users with samples of other kind of mammals (not shown on table V). Thus, nasal swab samples from cat were isolated on and identified by Maldi tof as *Pasteurella multocida* subsp. *multocida*. Moreover, pig samples gave isolation of pale pink colonies of *Actinobacillus pleuropneumoniae* (n=4), a bacterium responsible for a highly contagious disease widely distributed throughout major swine-raising countries.

3.2.2. Detection of *Pasteurellaceae* species in respiratory organ specimens

CHROMagar™ Pasteurella has been evaluated with veterinary clinical samples of diseased animal lungs. The results obtained are presented below.

Table V. Respiratory organ specimens tested on CHROMagar™ Pasteurella.

Laboratory, Country	Type of sample (# of samples)	Sensitivity	Specificity	Colony identification
Oniris site de la Chantrerie, Nantes, France	Lung samples Bovine (n=8)	100 %	100 %	API 20 NE
Arsia asbl., Ciney, Belgium	Lung samples Bovine (n=24)	83 % *P. multocida* (n=2) *M. haemolytica* (n=2)	100 % *E. coli* colonies are differentiated in bleu	Maldi tof
SEML Agrivalys 71, Service Bactériologie, Mâcon, France	Lung samples [§] Bovine (n=2) Sheep (n=2) Goat (n=2)	100 % *M. haemolytica* Bovine (n=1) Goat (n=2)	100 %	PCR, biochemical testing

Comparison with blood agar and Poly Vitex agar, 24h at 37 °C.

[§] On plates of routine media, some bovine and goat lung samples showed spread swarming of Proteus and only on CHROMagar™ Pasteurella, target species were easily detected.

According to the laboratories results, CHROMagar™ Pasteurella can be used to detect *Pasteurellaceae* species when testing respiratory organ specimens, such as unhealthy lung tissue from cattle and goats.

4. Conclusion

The performance of the CHROMagar™ Pasteurella medium has been validated by a series of evaluations. These evaluations included inclusivity and exclusivity studies, as well as analyses of respiratory tract samples and respiratory organ specimens.

Parameter	**Performance of CHROMagar™ Pasteurella**
Inclusivity/Exclusivity	100 % /100 % with bacterial & fungal strains.
Detection of *Pasteurellaceae* species	Sensitivity 91-100 % Specificity ~100 % Rare *E. coli* mauve colonies.
Morphological appearance of colonies	*M. haemolytica* generally develops in smaller colonies than *P. multocida*.
Microbial identification directly from colony	By Mass Spectrometry and PCR.

In appropriate storage, the shelf life of the powder base and supplements is > 18 months. Advantages in the selectivity and visualisation of colonies on CHROMagar™ Pasteurella plates compared to blood agar plates were reported from laboratories. Nevertheless, the blood agar plate provides valuable information and should not be omitted. Good preparation of CHROMagar™ Pasteurella can be verified by isolating recommended ATCC strains for Quality Control testing.

The results on CHROMagar™ Pasteurella plates are easy to read with the naked eye, strain confirmation can be carried out by biochemical testing, PCR or by mass spectrometry.

This medium has very good performances, but some limitations can be pointed out:

- *Pasteurellaceae* species cannot be distinguished from one another by colour.

Hugo CRUZ RAMOS, PhD.

Scientific Expert

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5. Literature

1) Bavananthasivam, J., Shanthalingam, S., Kugadas, A., Raghavan, B, Batra, S. and Srikumaran, S. 2018. β-hemolysis may not be a reliable indicator of leukotoxicity of *Mannheimia haemolytica* isolates. *Toxins*. **173:** 1-7.

2) Delano, M. L., Mischler, S. A., and Underwood, W. J. 2002. Biology and Disease of Ruminants: Sheep, Goats, and Cattle. *In Laboratory Animal Medicine*, 2nd Ed. Academic Press, p. 519-614.

3) Klima, C. L., Cook, S. R., Zaheer, R., Cook, S. R., Booker, C. W., Hendrick, S., Alexander, TR. W., and McAllister, T. A. 2014. Pathogens of bovine respiratory disease in North American feedlots conferring multidrug resistance via integrative conjugative elements. *J. Clin. Microbiol.* **52**: 438-48.

4) Tel, O. Y., Ötkün, S., Yücetepe, A. G., and Keskin, O. 2022. Investigation of the effectiveness of chromogenic media in the isolation of *Pasteurella multocida* and *Mannheimia haemolytica* from calf nasal swab samples. *Vet. Sci. Pract.* **17:** 81-86.

5) Wilson, B. A. and Ho, M. 2013. *Pasteurella multocida*: from Zoonosis to Cellular Microbiology. *Clin Microbiol Rev.* **26:** 631-55.

Annexes

Annex 1. Website information about CHROMagar™ Pasteurella.

Our Company Products Technical documents **CHROMagar™** The Chromogenic Media Pioneer Find your distributor Contact EN

Clinical Microbiology A complete range of culture media to help in clinical diagnosis

Food Industry A complete range of culture media for the food industry

Water Testing A complete range of culture media for water analysis

Veterinary Microbiology A complete range of culture media for the veterinary sector

CHROMagar™ Pasteurella

For detection of Pasteurellaceae

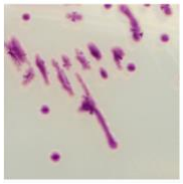
Order References

Please use these references when consulting your local distributor :

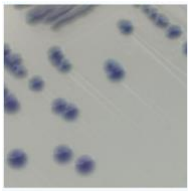
5000 mL Pack PR012

Included : base PR012(B) + supplement PR012(S1) + supplement PR012(S2)

Colony appearance



Pasteurellaceae
Pink to mauve



E. coli, coliforms
Inhibited or blue to steel blue

Composition

Powder Base	Total ..... 41.2 g/L Agar ..... 15.0 Salt ..... 5.0 Peptones ..... 21.0 Chromogenic and selective mix ..... 0.2 Storage at 15/30 °C - pH: 7.0 ± 0.2. Shelf Life ..... > 18 months
Supplement S1 (Included in the pack)	Liquid form ..... 8 mL/L Storage at 15/30 °C Shelf Life ..... > 18 months
Supplement S2 (Included in the pack)	Powder form ..... 1 g/L Storage at 2/8 °C Shelf Life ..... > 18 months
Usual Samples	nasal swab, trans-tracheal aspiration (TTA), bronchial lavages, lung.
Procedure	Isolation & spreading method. Incubation 18- 24 h, 37 °C. Aerobic conditions.

Performance

In the veterinary field, respiratory infections in cattle herds can cause stunted growth, decline in milk production and death of animals, leading to significant economic losses. Bacteria of the Pasteurellaceae family are commensal to the upper respiratory tract of many vertebrate species. Among them, *Histophilus somni, Pasteurella multocida* and *Mannheimia haemolytica* are among the main bacteria associated with the pathogene complex of bovine respiratory diseases. During infection, these species cause complications which can lead to sepsis and death of the animal.

CHROMagar™ Pasteurella was therefore developed to improve the detection of Pasteurellaceae from a bovine respiratory sample (eg. nasal swab, trans-tracheal aspiration (TTA) and lung). This chromogenic medium to aid in qualitative diagnosis allows the detection and isolation of Pasteurellaceae colonies by inhibition or differentiation of the annex flora.

CHROMagar™ Pasteurella medium can also be used for samples from the respiratory tract of mammals such as cats and dogs.

1. Unique chromogenic media : First and only available specific medium for Pasteurellaceae in the market

2. Lower workload contrary to general media like blood agar, CHROMagar™ Pasteurella differentiate the target group from the other bacteria by a distinctive mauve colony color.

4. Early plate reading : Most isolates will have a noticeable colony size after just overnight incubation.

3. Highly selective : Most of the environmental flora will be inhibited.