Evaluation of the Test Characteristics of the HyMast® Bacteriological Test System

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Introduction

Mastitis continues to be a disease of major economic importance to the dairy industry, despite the wide use of mastitis control programs. Approaches to mastitis therapy may range from conservative to aggressive, depending on cow and management factors. Knowledge of the organism, prior to treatment, would appear to be of benefit in selecting the most appropriate course of therapy. The HyMast® test (Pharmacia & Upjohn, Kalamazoo, Michigan) is a selective media bacteriological test system for detection of gram-positive (*Staphylococci, Streptococci*) and gram-negative (coliform) organisms in milk. Although the primary use of this test has been for decision making in mastitis therapy, there is interest in identifying specific organisms from the test. The objectives of this study were to evaluate the sensitivity and specificity of the HyMast test read by producers on-farm and by readers in-clinic at 3 time periods, compared to standard milk bacteriology.

Materials and Methods

Dairy producers participating in a clinical mastitis decision-making field study and 6 individuals from the Ontario Veterinary College were asked to determine the presence or absence of bacterial growth (gram-positive and/or gram-negative) at 12, 24 and 36 hours post-set up. For the in-clinic portion of the trial, if growth was present, readers were asked to identify the specific organism based on the HyMast package insert and interpretative colour chart. *Staphylococci / Streptococci*-select coliform HyMast tests were used.

Milk samples used for the study were collected from clinical mastitis cases and newly elevated somatic cell count (SCC) cows, (threshold >200,000 cells/ml based on most recent Dairy Herd Improvement (DHI) test). Laboratory culture techniques were used to plate milk samples in accordance with National Mastitis Council (NMC) recommendations.[1]

Results and Discussion

Results for the in-clinic portion of the trial were as follows: A total of 206 HyMast tests were examined. Ninety-one percent of the tests were from high SCC cases and 9% were from clinical mastitis cases. Based on standard milk bacteriology, the prevalence of gram-positive growth in the test population was 76%, gram-negative growth 4% and no growth 12%.

At 12 hours, the sensitivities for gram-positive growth were low for all readers (Table 1). In other words, the ability to correctly identify gram-positive growth varied between 26% and 58%. Approximately 40% to 70% of the samples truly had growth, but at that time there was no growth or the readers were unable to identify it

Table 1. Sensitivity and specificity of the HyMast test for gram-positive growth

Reader ID	Sensitivity / Specificity (%) 12 hrs		24 hrs		36 hrs	
	Sensitivity	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
1	40 (29,50)*	74 (54,94)	45 (33,58)	80 (55,100)	80 (71,90)	39 (16,61)
2	36 (28,43)	84 (72,97)	72 (64,80)	47 (29,65)	88 (82,93)	44 (26,63)
3	58 (50,66)	61 (44,78)	75 (68,82)	48 (31,66)	91 (86,96)	30 (12,47)
4	36 (28,44)	80 (66,94)	62 (54,70)	77 (62,92)	89 (84,95)	44 (23,64)
5	31 (24,39)	86 (74,99)	63 (55,71)	56 (37,74)	87 (80,93)	36 (16,57)
6	26 (18,34)	90 (77,100)	51 (42,61)	65 (44,86)	87 (80,94)	45 (23,67)

*95% confidence limits

on the HyMast tests. The specificities (the ability to correctly identify gram-negative or no growth) for all readers were high. Therefore, there were few false-positive results. At 24 hours the sensitivities improved while the specificities decreased. At 36 hours all readers had high sensitivities, but there were now more false-positive results. These results suggest that treatment decisions at 12 hours of incubation would fail to identify a large number of positive cases. On the other hand, waiting 36 hours would correctly identify most of the positive cases but would also result in the unnecessary treatment of some gram-negative or no growth cases.

The prevalence of major contagious pathogens (*Staphylococcus aureus, Streptococcus agalactia*) in the test population was 14%. At 12 hours, the sensitivities (the ability to correctly identify a major contagious pathogen if present) were quite low and less consistent between readers (Table 2). With time the sensitivity improved, but still was low compared to the ability to correctly identify gram-positive growth. At 36 hours, false-negative results varied between 33% and 56%, while false-positive results varied between 31% and 43%. In herds trying to identify cows with contagious mastitis, these misclassifications could be costly.

Based on these results, the HyMast test is useful for determining gram-positive growth versus gram-negative or no growth, when the test is read at 36 hours of incubation. Decisions based on results obtained at earlier incubation times (particularly at 12 hours) will result in the misclassification of some cases. The low sensitivities for identifying specific organisms makes the test inappropriate for determining the exact cause of the intramammary infection by direct visual inspection alone. Further microbiological testing of colonies isolated from HyMast paddles was not evaluated in this study.

References

1. National Mastitis Council. 1987. Laboratory and field handbook on bovine mastitis. Hoard and Sons Co., Fort Atkinson, WI.

Table 2. Sensitivity and specificity of the HyMast test for major contagious pathogens.

Reader ID	Sensitivity / Specificity (%) 12 hrs		24 hrs		36 hrs	
	Sensitivity	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
1	36 (11,61)*	87 (81,94)	25 (1,50)	86 (77,94)	67 (40,93)	68 (57,78)
2	28 (10,46)	86 (80,91)	35 (14,56)	78 (71,85)	47 (25,70)	69 (60,77)
3	8 (0,19)	87 (82,93)	28 (10,46)	74 (67,81)	44 (21,67)	64 (55,72)
4	0 (0,0)	95 (92,98)	45 (23,67)	78 (72,85)	58 (36,80)	57 (48,66)
5	32 (14,50)	91 (87,96)	26 (7,46)	87 (81,93)	46 (16,75)	71 (63,79)
6	5 (0,14)	98 (96,100)	18 (2,34)	83 (76,90)	46 (16,75)	61 (51,70)

*95% confidence limits