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Abstract
In the present study, the results of a 3M molecular detection system (3M MDS) prototype were compared to qualitative methods for the detection of *Salmonella* Enteritidis (DIN EN ISO 6579) and *Listeria monocytogenes* (DIN EN ISO 11290-1). The 3M MDS test is based on a combination of isothermal DNA amplification and bioluminescence detection after enrichment of microorganisms. The use of isothermal DNA amplification in combination with bioluminescence detection leads to highly sensitive results. The total testing time in MDS is 75 min after enrichment of samples for 18 - 48 h. A total of 155 naturally contaminated and artificially inoculated samples (meat, delicatessen, spices, milk powder and cacao powder) were tested on *Salmonella* Enteritidis and *Listeria monocytogenes*. With the exception of mixed spices no differences could be observed between 3M MDS testing and the results of standard DIN EN ISO methods.

Comparison of a 3M Molecular Detection System prototype with the DIN EN ISO-methods for the detection of *Listeria monocytogenes* and *Salmonella* Enteritidis

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Introduction and Purpose

The 3M Molecular Detection System (3M MDS) is an automated system for the rapid detection of pathogenic organisms, such as *Salmonella*, *Listeria* and *E. coli* O157:H7. The system was developed to detect pathogens in different foods like meat, convenience food, fruits, vegetables, pet food, and surface swabs. Due to the combination of isothermal DNA amplification and bioluminescence detection it is possible to achieve highly sensitive results.

In the present study, the results of an automated 3M MDS prototype (Fig.1) were compared to the results achieved by using qualitative DIN EN ISO methods for the detection of *Listeria monocytogenes* and *Salmonella* Enteritidis in meat, delicatessen, spices, milk powder and cacao powder.

Fig.1: 3M MDS device and test assay (Picture: 3M, St. Paul, USA)

Material and Methods

A total of 155 samples (72 naturally contaminated and 83 artificially inoculated with 10^2 cfu/g) were tested to determine *S.* Enteritidis (DIN EN ISO 6579) and *L. monocytogenes* (DIN EN ISO 11290-1) (Tab.1). The enrichment of samples as well as the testing in 3M MDS was carried out by double testing. The overall testing time with the automated system was 75 min after enrichment for 18 hours – with detection of the samples in real-time.

The device is easy to use and samples may be prepared in only few steps (Fig.2). For the determination of the detection limit, samples were inoculated with different bacterial counts (10^2 to 10^5 cfu/g) and tested without enrichment. Accuracy, specificity and sensitivity of the 3M MDS were determined according to DIN EN ISO 16140.

Tab.1: Numbers of analyzed foods and parameters.

Parameter / Product	*Salmonella*	*Listeria*
Poultry	15 (3)	15 (3)
Marinated poultry	4	4
Spices / mixed spices	25 (16)	25 (16)
Delicatessen	24 (14)	24 (14)
Ground meat	4 (3)	4 (3)
Milk powder	2 (2)	3 (3)
Cacao powder	3 (3)	3 (3)
Number of samples	**77 (41)**	**78 (42)**
Total number of tests	**174**	**172**

Numbers in brackets: inoculated samples.

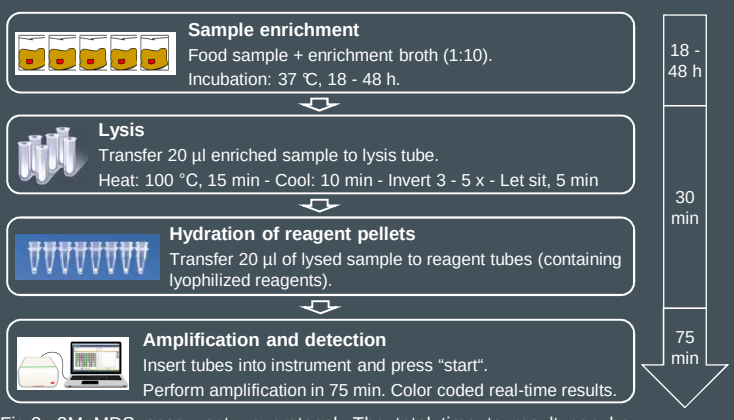

Fig.2: 3M MDS assay set up protocol. The total time to result can be achieved in less than 20 h compared to 90 - 96 h for DIN EN ISO methods.

Results

The inoculation tests showed, that even low bacterial counts (10^2 cfu/g) could be detected as positive in 20 - 30 min after enrichment for 18 h (examples in Fig.3).

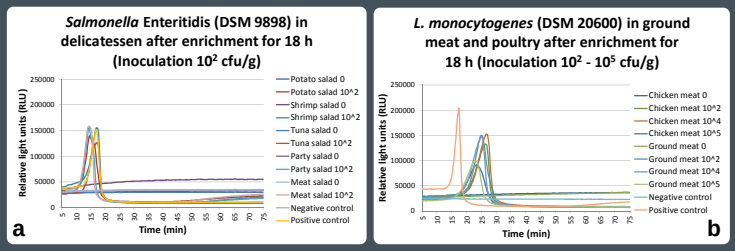

Fig.3: Detection of *S.* Enteritidis in delicatessen (a) and *L. monocytogenes* in ground meat and poultry (b) after enrichment for 18 h.

No differences were found between 3M MDS testing and the results of the standard qualitative methods for the detection of *S.* Enteritidis (DIN EN ISO 6579) and *L. monocytogenes* (DIN EN ISO 11290-1) in meat, delicatessen, milk powder and cacao powder. Differences between the two methods could be observed while testing mixed spices (Tab.2). The mixed spices which showed false-negative results in 3M MDS contained ingredients like celery, mustard or paprika. In further inoculation experiments with same spices, the sample enrichment was varied by performing 1:100 dilutions. In these experiments all inoculated samples (10^2 cfu/g) were tested as positive (data not shown). Experiments for the determination of the 3M MDS detection limit in meat and delicatessen showed that for positive detection without enrichment a bacterial count of 10^5 cfu/g was required. This leads to the conclusion that for reliable results the enrichment step for a minimum of 18 h is essential.

The automated molecular detection system was compared to the standard DIN EN ISO methods according to DIN EN ISO 16140. The results were 98 % relative accuracy, 100 % relative specificity and 96 % relative sensitivity for *S.* Enteritidis. For *L. monocytogenes*, the results showed 96 % relative accuracy, 98 % relative specificity and 95 % relative sensitivity.

Tab.2: Detection of *S.* Enteritidis and *L. monocytogenes* in naturally contaminated and artificially inoculated products. Comparison of the results generated by 3M MDS testing and DIN EN ISO methods for *Salmonella* (6579) and *L. monocytogenes* (11290-1).

Test	*Salmonella*			*Listeria*		
Products	**Number of tests**	**Deviation MDS + ISO -**	**Deviation MDS - ISO +**	**Number of tests**	**Deviation MDS + ISO -**	**Deviation MDS - ISO +**
Poultry	28	0	0	30	0	0
Marinated poultry	8	0	0	8	0	0
Spices / mixed spices	37	0	2	37	1	3
Delicatessen	34	0	0	34	0	0
Ground meat	8	0	0	8	0	0
Milk powder	2	0	0	1	0	0
Cacao powder	3	0	0	4	0	0
Total	**120**	**0**	**2**	**122**	**1**	**3**

Conclusion

Compared to the DIN EN ISO methods, the 3M MDS showed reliable results in reduced time for the detection of *L. monocytogenes* and *S.* Enteritidis in foods. False-negative results which occurred while testing mixed spices could be eliminated by variation of sample enrichment.