

Abstract

Mycoplasmas are the smallest and simplest self-replicating organisms, some of which can be pathogenic in humans, for example, causing pneumonia. While they are frequent contaminants of cell cultures, the contamination can have no visible sign in cultures, as mycoplasmas can adhere to the cell surface without killing the cells and causing turbidity in the medium. They are also too small to be observed by optical microscopy. Moreover, current mycoplasma detection methods are mostly time-consuming and laborious. Thus, in this study, we verified the usefulness of an isothermal PCR-based rapid mycoplasma detection kit developed by InvivoGen, **MycoStrip™**, and compared its detection sensitivity with other detection methods. In the experiment, *M. hyorhinis* (NBRC 14858) was added to **VERO cells (JCRB0111)** for culturing, the diluted samples were tested by MycoStrip™, **bioluminescence assay**, **fluorescent staining** and **nested-PCR** respectively. It was confirmed that the sensitivity of MycoStrip™ is equivalent to that of fluorescent staining, showing a sufficient detection efficiency. We demonstrated that the isothermal PCR-based MycoStrip™ is a simple and highly versatile mycoplasma detection method, considering that the assay does not require special equipment, and that the results can be obtained easily and quickly by mixing the reagent with the sample and dropping it on the strip. We envision MycoStrip™ could be universally used for routine detection of mycoplasma contamination.

Background

Japanese Pharmacopoeia

Method A: Culture Method

A detection method to grow mycoplasma directly on selective media

Method B: Indicator Cell Culture Method

A detection method to stain mycoplasma genomic DNA

Method C: Nucleic Acid Amplification Test (NAT)

A detection method to amplify mycoplasma specific DNA by PCR test

Other methods include bioluminescence assay, etc.

The advantages and disadvantages of different detection methods	Advantage		Disadvantage		
	Culture Method	Relatively high sensitivity; able to detect many kinds of mycoplasmas	Easy and fast	Time-consuming; complicated culture conditions	Low sensitivity
	Bioluminescence Assay	Easy and fast	High sensitivity	Time-consuming	
	Indicator Cell Culture Method	High sensitivity	High sensitivity	Detect limited species of mycoplasma	
	Nucleic Acid Amplification Test	High sensitivity			

Each method has its own advantages and disadvantages. Their detection limits differ.



The combined use of different detection methods is important to prevent an oversight of contamination.

A simple, rapid and highly versatile detection method is needed

Method

Experimental materials

● InvivoGen's MycoStrip™ (Cat No. rep-mys-10)

From InvivoGen HP
From Nacalai Tesque, Inc. HP

※ Provided by Nacalai Tesque, Inc.

4 EASY STEPS TO DETECT MYCOPLASMA IN YOUR CELL CULTURES

- 1 PREPARE YOUR SAMPLE**
Mix prepared test sample (Culture supernatant after centrifugation) with Reagent Mix and Reagent Buffer
- 2 INCUBATE AT 65°C FOR 40 MINUTES**
Use a thermocycler at 65°C heat for 40 mins (Perform Isothermal PCR)
- 3 STOP THE REACTION AND LOAD THE CASSETTE**
Mix Migration Buffer with warmed sample and transfer to Strip
- 4 READ YOUR RESULTS WITHIN 2-5 MINUTES**
Add to Strip Wait 2-5 minutes for interpretation

CELL CULTURE CONTAMINATION 95%

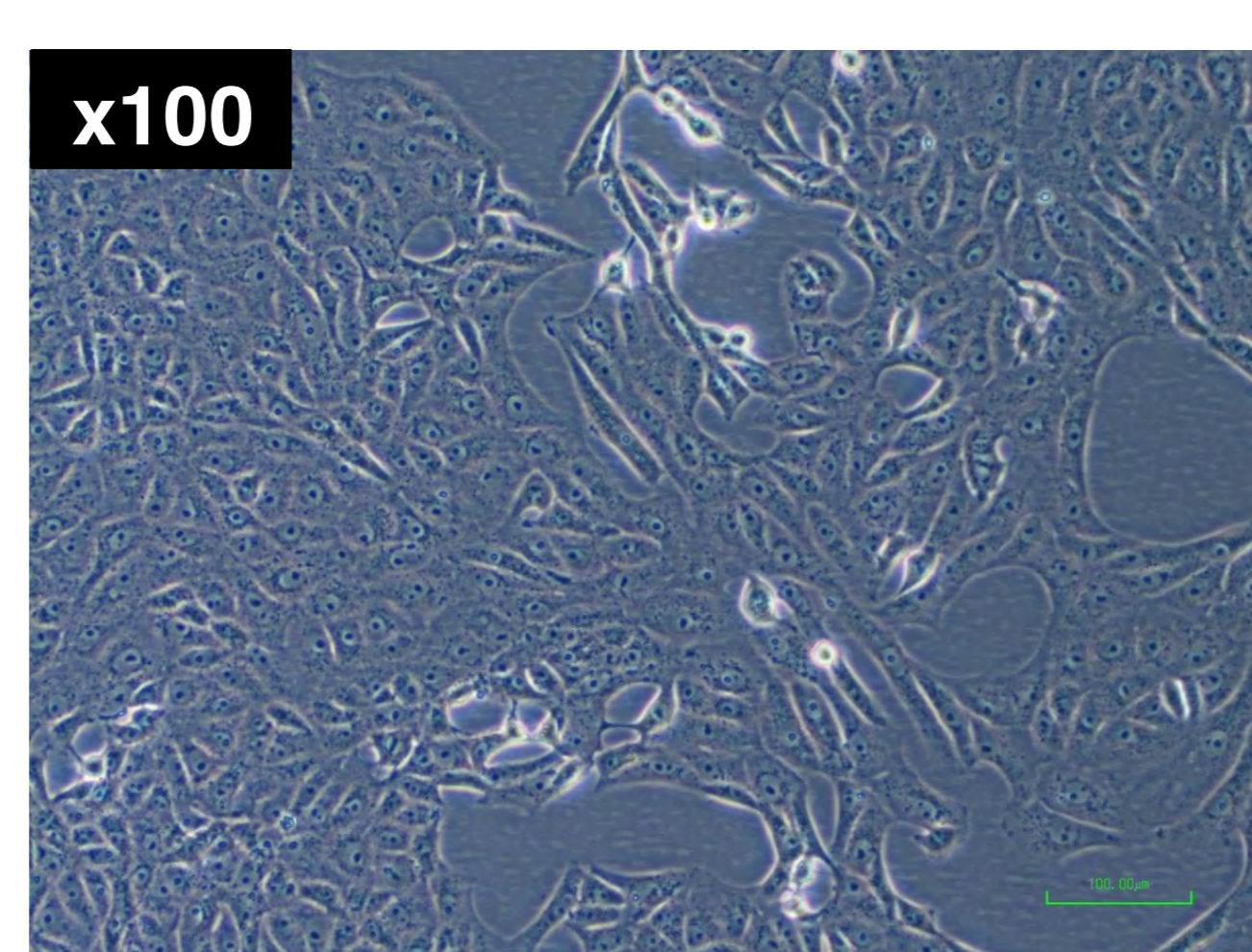
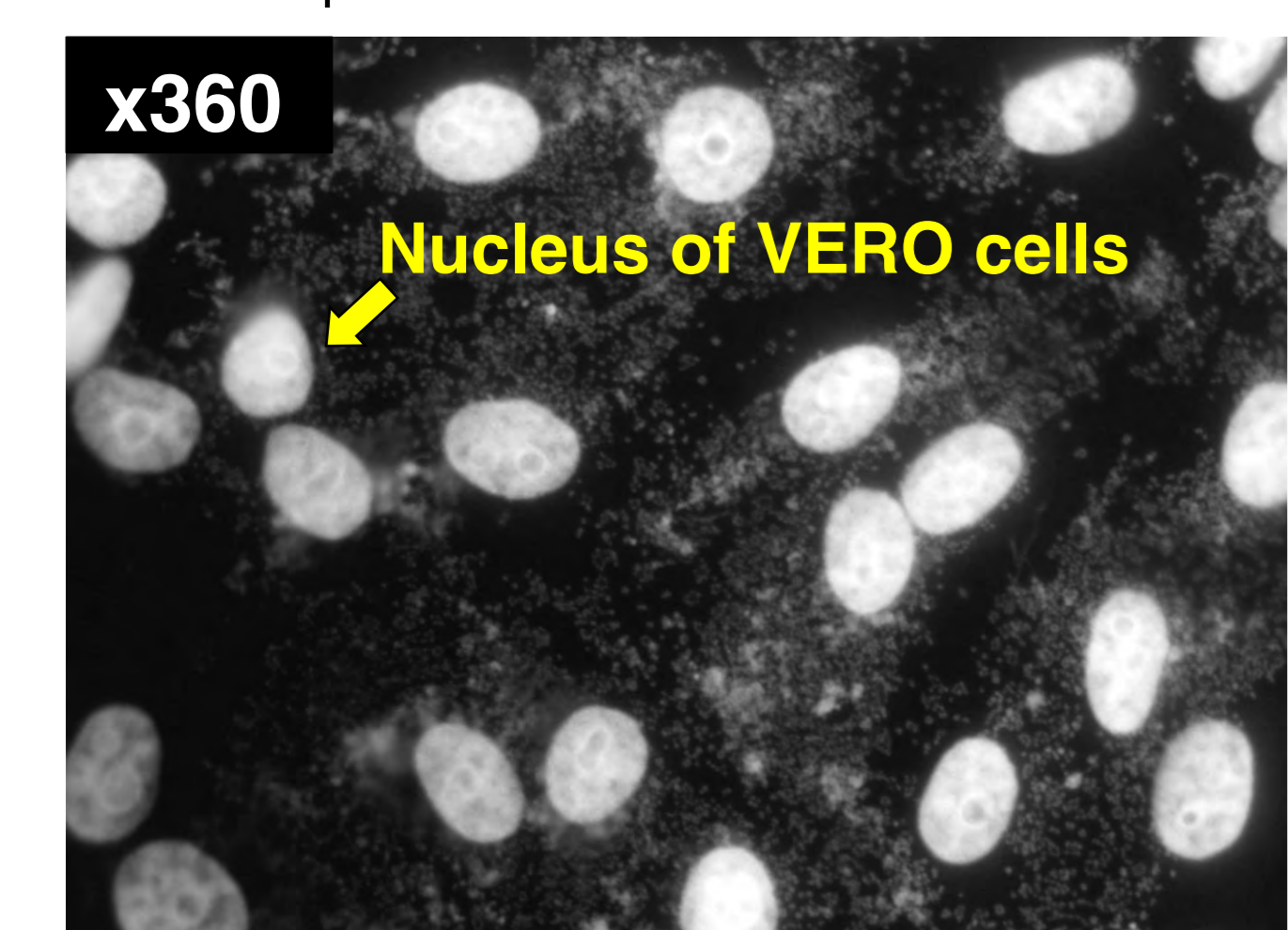
MYCOPLASMA:
M. orale
M. hyorhinis
M. arginini
M. fermentans
M. hominis
ACHOLEPLASMA:
A. laidlawii

MycoStrip™ with cassette vs **MycoStrip™ without cassette**

Positive: C, T, SP, CP bands visible
Negative: C, T bands visible

● Indicator cells: VERO (JCRB0111)

* African green monkey kidney-derived cell line
* Deletion of interferon α
(Prone to microbial infection due to immunodeficiency)



Comparison

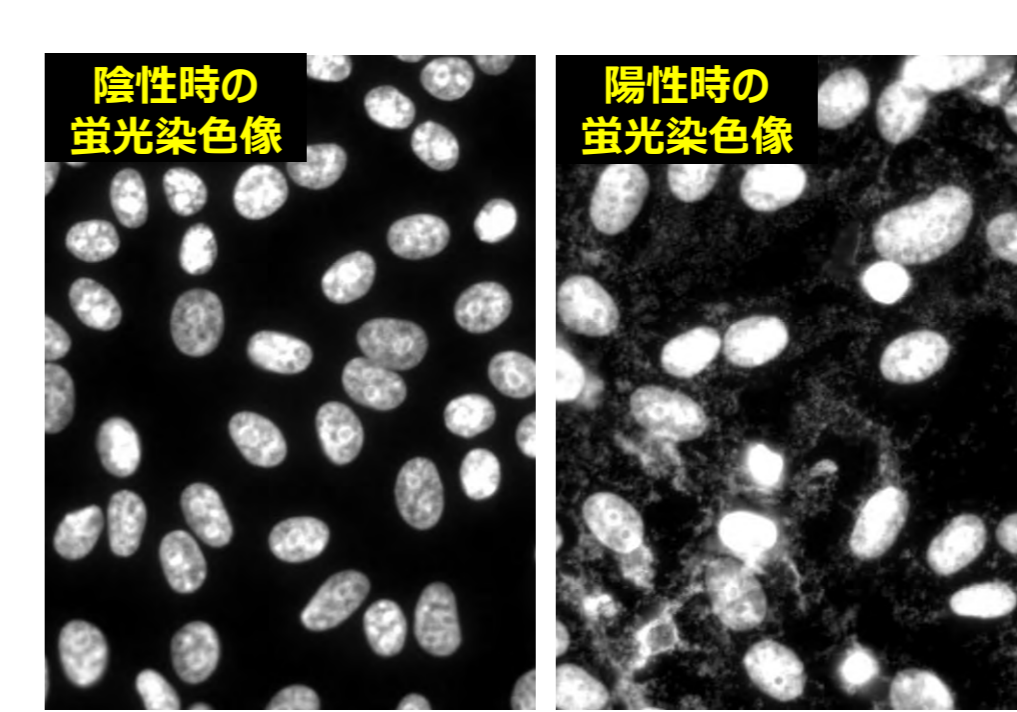
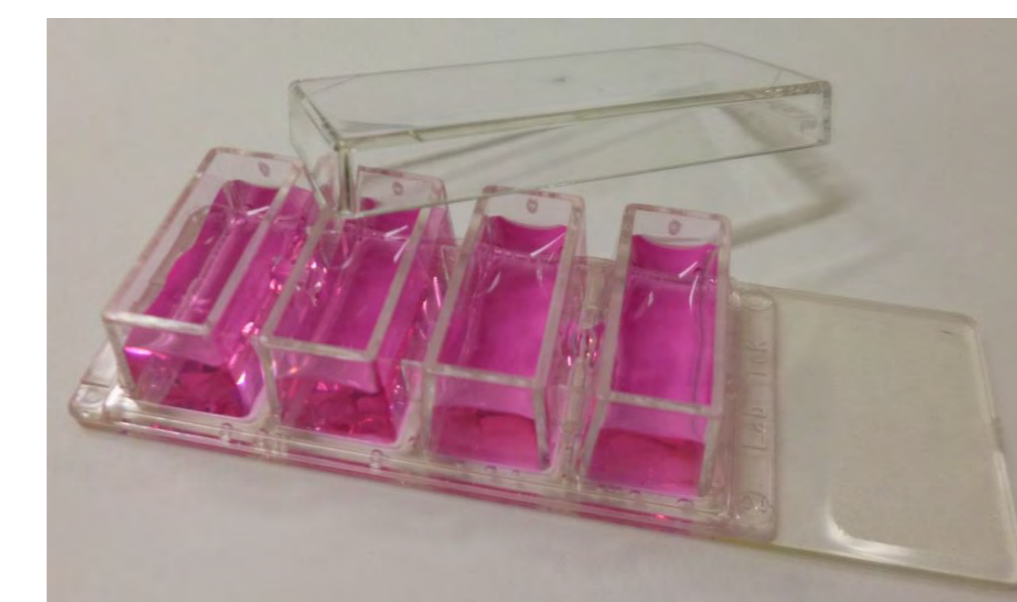
Bioluminescence assay

● MycoAlert



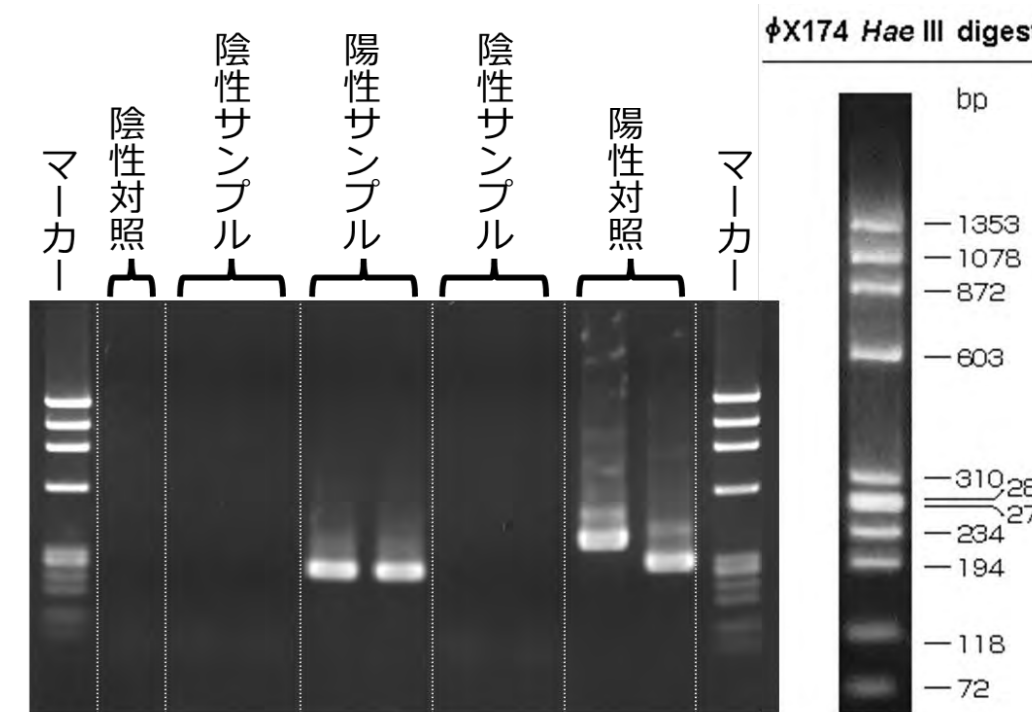
Fluorescent staining

● Hoechst33258



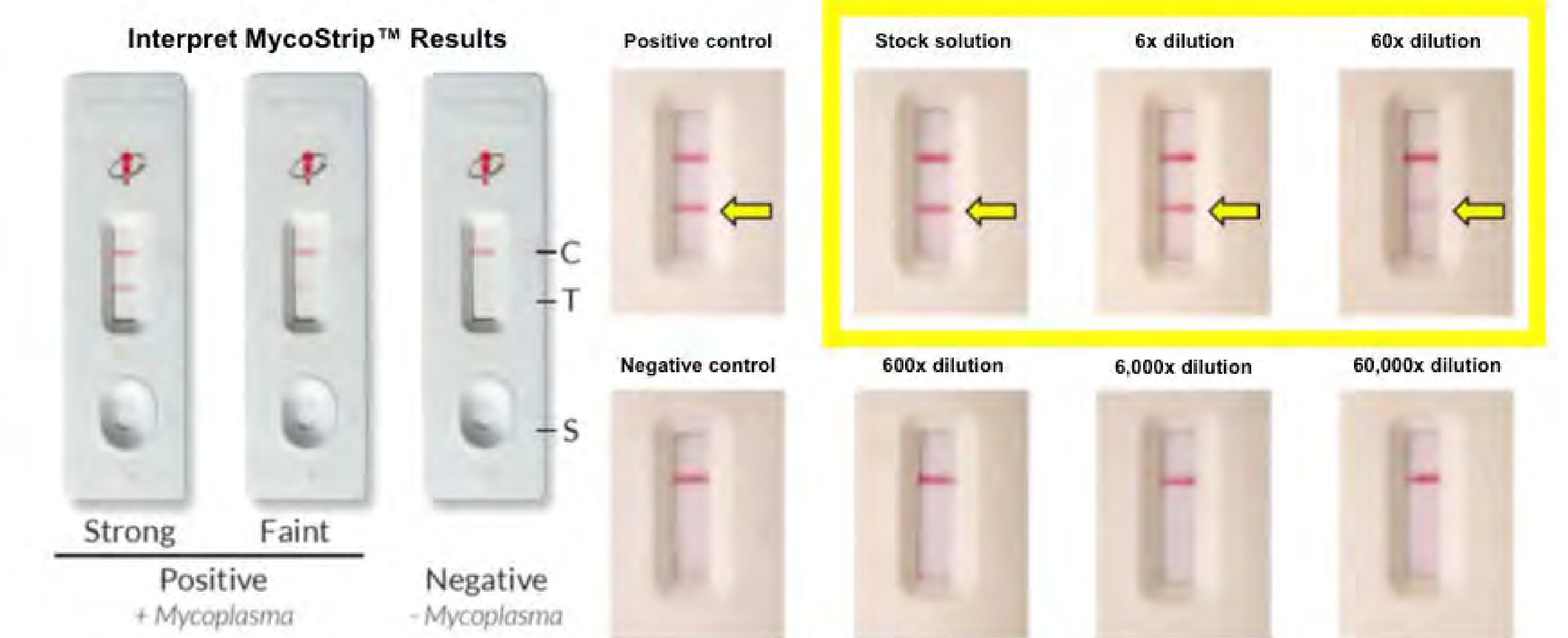
Nucleic acid amplification

● Nested-PCR



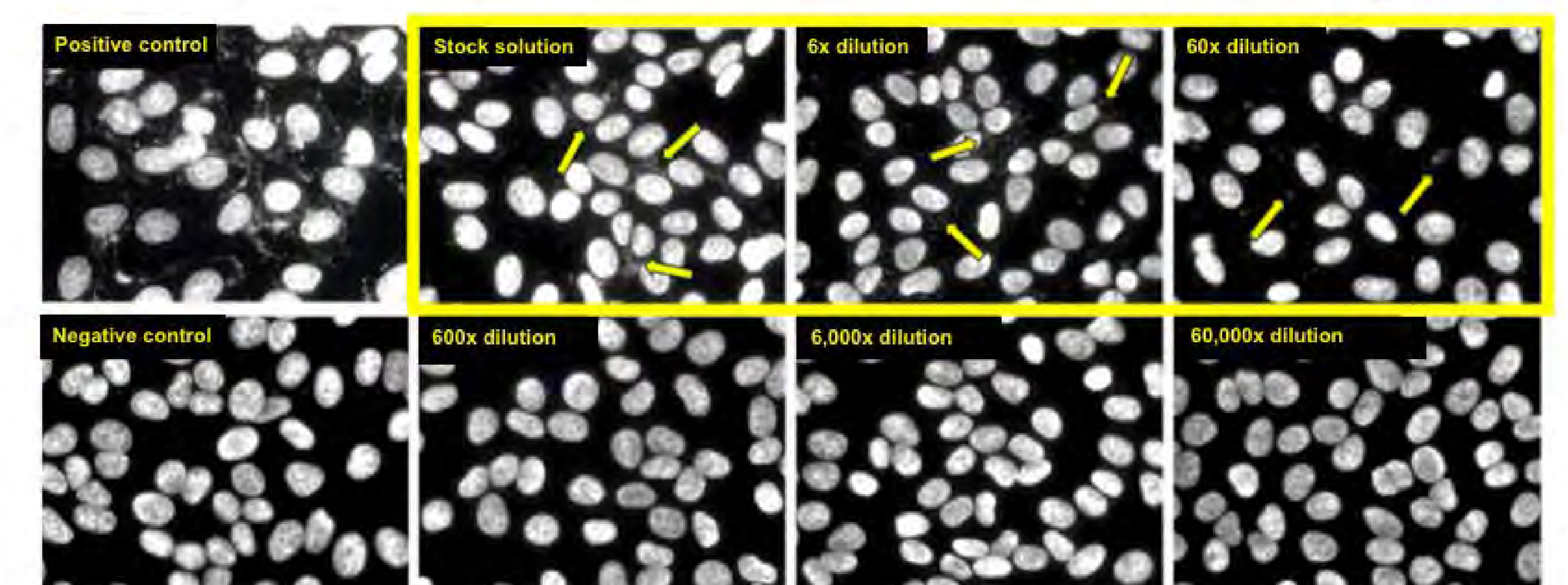
Results

● MycoStrip™



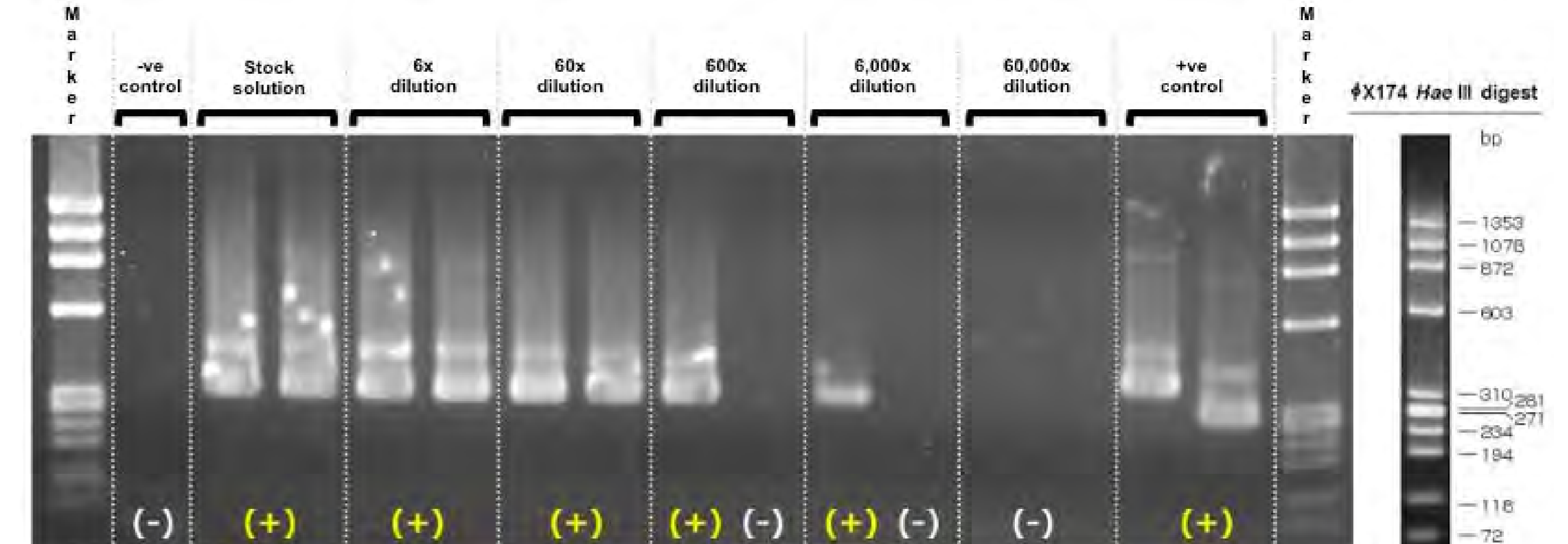
Mycoplasma contamination was detected at up to 60-fold dilution. Same sensitivity as the fluorescent staining method.

● Hoechst 33258 staining



Mycoplasma contamination was detected at up to 60-fold dilution.

● Nested-PCR



Detected up to 60-fold dilution of the mycoplasma-containing sample. Mycoplasma contamination was detected with a probability of 50% at 600-fold and 6,000-fold dilutions.

Summary

	MycoStrip™	MycoAlert	Fluorescent Staining	Nested-PCR
Stock solution of the cell culture supernatant	○ (1/1)	○ (5/5)	○ (1/1)	○ (2/2)
6x dilution	○ (1/1)	○ (1/1)	○ (1/1)	○ (2/2)
60x dilution	○ (1/1)	X (0/1)	○ (1/1)	○ (2/2)
600x dilution	X (0/1)	X (0/1)	X (0/1)	△ (1/2)
6,000x dilution	X (0/1)	X (0/1)	X (0/1)	△ (1/2)
60,000x dilution	X (0/1)	X (0/1)	X (0/1)	X (0/2)

InvivoGen's MycoStrip™ has a sensitivity about 10 times higher than that of MycoAlert, a bioluminescent assay that allows a simple and fast (about 30 mins) detection. In addition, MycoStrip™ has a similar sensitivity with the detection methods described in the Japanese Pharmacopoeia, i.e. fluorescent staining and nested-PCR.

Conclusion

- MycoStrip™ does not require special equipment
- Sensitivity of MycoStrip™ is equivalent to that of the mycoplasma test listed in the Japanese Pharmacopoeia

MycoStrip™ is well suited for routine detection