No.	Category 1	Category 2	Question	Answer
PCR systems and analysis				
1-3	Fundamental item	Tools/devices	Other than the kit, what is required?	The required items are: real-time PCR system (dual wavelength compatible), DNA extraction kit, micropipette, filter chip (sterilized DNase/RNase, Free), mixer, heat block, centrifugal separator (20,000 x g compatible), and 1.5 mL microtube.
1-4	Fundamental item	Tools/devices	What real-time PCR system fluorescent wavelength is required for detection?	Dual wavelength (FAM and HEX or ROX) fluorescent wavelength is required for mycoplasma gene detection of this kit, which uses the Taqman probe method. Please confirm that the real-time PCR system you are using can perform fluorescent detection in combination with FAM + HEX or FAM + ROX.
	Fundamental item	Detection	Where is the target area?	It is set to bacterial 16S rRNA genes and the spacer area.
			Can the Myco Finder only measure the 7 strains specified by the Japanese Pharmacopoeia?	It is understood that it can measure 142 strains in silico.
2-23	Operation	Detection	How should we configure the settings of the real-time PCR system?	The settings are different for each device, so please carefully read the operational manual of the system before configuring it. If you can choose between NORMAL mode and FAST mode, please select FAST mode.
2-24	Operation	Detection	We cannot set the device that I am using to 3 seconds or 1 second. What should we do?	Please set the time to the smallest number that can be set in the device that you are using. For some devices, reference programs are listed on our company's homepage: https: //cosmokai.com/img/products/myco/MycoFinderSample2.pdf
2-25	Operation	Detection	How are positives and negatives judged? Is there a setting for a cut-off value?	For judgments, we recommend automatic judgment using the analysis software of the device. (If you can set any of the following methods in the analysis screen of the software you are using, please select them: Regression method, Background subtracted Curve Fit mode, and 2nd Derivative Maximum method.

No.	Category 1	Category 2	Question	Answer
Nucleic acid extraction				
1-10	Fundamental item	Other	With regards to the kit from QIAGEN, what are the stability, time period, and price (and running costs)?	The stability when stored at room temperature is 1 year. For temperatures of 25 degrees Celsius or higher, cryopreserving the included Proteinase K is preferable. For the price, please contact QIAGEN or one of their distributors.
2-6	Operation	Samples	What is the upper limit for the number of cells that can be used in tests?	In kit validation, tests are conducted using samples of DNA extracted from 1 CHO-DG44 cell × 10^6/mL. The amount of DNA that can be obtained from extraction depends on the type of cell, so you will need to investigate the optimal number of cells for the type of cell that you will use.
2-7	Operation	Samples	Is it possible to perform tests using culture supernate?	Mycoplasma infects culture cells, so only a certain percentage of the contamination is present in the culture supernate. Therefore, there is the risk of it not being possible to obtain a sufficient sensitivity when using a culture supernate as a sample. When using a culture supernate, it is thought that it is necessary to investigate conditions, of concentration and the like, that will make it possible to obtain sufficient sensitivity by means of centrifugal separation.
2-8	Operation	Samples	What are the concentration conditions of samples?	In order to remove mycoplasma bacterial cells in cell suspensions, we recommend performing centrifugal separation at 15,000 rpm (20,000 x g) for 10 minutes. After concentration, please resuspend using PBS (-) or the like and perform DNA extraction according to the protocols.
2-9	Operation	Samples	Can skin tissue or the like be used as samples?	Yes, they can, but the method of DNA extraction will be different.
2-10	Operation	Extraction	Is it possible to use other methods during the DNA extraction process?	It is possible to perform DNA extraction using general DNA extraction methods of kits from other companies. However, we recommend that you confirm the sensitivity. Our company uses the QIAamp UCP DNA Micro Kit from QIAGEN to perform kit validation.
2-11	Operation	Extraction		Please double check the protocols of the extraction kit that you are using. Please confirm that there is no precipitation of the reagent that you used, and that you have added the necessary amount of reagent, etc. to be used after adding the ethanol, etc.

No.	Category 1	Category 2	Question	Answer
2-12	Operation	Extraction	We cannot perform extraction correctly. Also, the volume is remarkably small.	It is thought that ingredients contained in the culture medium can have an effect on the extraction efficiency. This problem can be fixed by replacing the culture medium with PBS after performing centrifugal separation on the cell suspension.
2-13	Operation	Extraction		The extraction efficiency decreases remarkably if there is an excessive number of cells in the sample. Please use the appropriate number of cells and perform extraction again.
2-14	Operation	Extraction	We could not obtain nucleic acids after adding 10 CFU of mycoplasma to a solution that does not contain any cells, such as a culture medium or physiological saline solution, and then performing nucleic acid extraction. What is the cause of this?	It is thought that it is possible to extract nucleic acids contained in samples at or below the extractable amount of the extraction kit. If you estimate that the amount of nucleic acids obtained by the QIAamp UCP DNA Micro Kit, which is recommended by our company, will be 10 ng or less, it is recommended that you use carrier DNA.
Kit specifications				
1-5	Fundamental item	Kit specifications	Are there longer versions of the strip tubes?	There are only short strip tubes. If you are using equipment that only supports long ones, you use it by dissolving the reagent in the sample using strip A => strip B => long tube, and then transferring all of it to the long tube.
1-6	Fundamental item	Kit specifications	Is it possible to determine the strain from the detection results?	This kit amplifies and detects DNA sequences which are common among many types of mycoplasma, etc., so it is not possible to determine the strain from the detection results.
1-7	Fundamental item	Kit specifications	Is it possible to quantify the mycoplasma by using the included positive control and making a calibration curve?	This kit was developed as a qualitative kit, so the quantitivity is not assured.

No.	Category 1	Category 2	Question	Answer
1-8	Fundamental item	Kit specifications	What is the expiration date after opening a product?	As long as there is no contamination of DNase or the like after opening, you can use the product by the expiration date.
1-9	Fundamental item	Kit specifications	Is it possible to cryopreserve the product?	No, it is not. Please refrigerate the product.
2-16	Operation	Measurement	Is it possible to use a strip that has been cut?	It is possible to use a strip that has been cut. In such an event, please set an empty PCR tube in an empty space to maintain balance when supplying to the PCR system. If it cannot be balanced on the plate, there is the risk of this affecting temperature measurement and making it impossible for PCR reactions to occur correctly.
Japanese Pharmacopoeia validation				
1-1	Fundamental item	Japanese Pharmacopoeia relatec	Are there rules regarding the intervals between validations?	There are no rules concerning the frequency of validations.
1-2	Fundamental item	Japanese Pharmacopoeia relatec	What conditions must be fulfilled in order to use a substitute for A method or B method?	For the 7 strains of mycoplasma specified by the Japanese Pharmacopoeia, it must be indicated that detection of 10 CFU/mL in A method and 100 CFU/mL in B method is possible through the performance of equivalence tests. However, this kit has already been fully validated, so, if you are using it, you can download the basic data from our homepage. (For detailed data, please contact a sales representative.) Also, depending on the user, equipment validation of the product may be required.
2-1	Operation	Test methods	What should be done for equipment validations and for validations by cell?	It is necessary to confirm that 10 CFU/mL of the 7 strains of mycoplasma specified by the Japanese Pharmacopoeia can be accurately and reproducibly detected by equipment and by cell. Moreover, it is thought that it is not necessary to calculate the detection sensitivity by means of statistical analysis while using a three-fold serial dilution. Reference 1

No.	Category 1	Category 2	Question	Answer
2-2	Operation	Test methods	When performing equipment validation, is there a specific number of times that tests must be conducted?	The Japanese Pharmacopoeia doesn't have any specific rules concerning the number of tests conducted, but please confirm that the number of tests is sufficient for the purpose of use of the device and that the performance needed for the purpose of use can be obtained. For official information, you can check with PMDA.
2-3	Operation	Test methods	Are there internal controls (internal standards)?	The positive control of this kit can be evaluated by extracting and measuring a sample of the sample (200 uL) before DNA extraction. The positive control includes positive control specific sequences other than mycoplasma gene sequences, and it is possible to distinguish with mycoplasma genomic DNA by detecting two types of fluorescence: FAM and ROX (or FAM and HEX).
2-5	Operation	Test methods	Where can standard mycoplasma strains be acquired?	Currently, we don't have the 7 standard strains specified as subject to detection in the Japanese Pharmacopoeia 17th Edition, but it is possible to purchase the 7 strains for which the CFU/mL are known from the American Type Culture Collection (ATCC) or another source. Also, users culture and make strains purchased through the National Institute of Technology and Evaluation Biological Resource Center (NBRC), etc.
			Culture method of mycoplasma strains	This information is noted in the full kit evaluation.
			What is the CFU (Colony Forming Unit) measurement method?	This information is noted in the full kit evaluation.
			What are the GC/CFU measurement methods?	This information is noted in the full kit evaluation.
			Is there a permissible range for GC/CFU values?	It has been said that the permissible range of QC/CFU values is from 1 to 100.
			Is it possible to substitute the 7 standard product strains (viable bacteria) used in equipment validation with, for example, bacteria-derived genomic DNA, plasmid DNA, or the like?	Equipment validation by the Japanese Pharmacopoeia has only been confirmed for viable bacteria.
			What is the dilution method of standard products?	It can be done using sterile physiological saline solution, but we recommend PBS (D-PBS- Code: 045-29795WAKO) or the like, which have been tested for mycoplasma.
			We cannot obtain the required detection sensitivity.	Increase N. For example, if N=4, then it will be judged as positive in the event that 1/4 is positive.
			The number of cells of the test reagent is sometimes 1 × 10^6/mL or higher.	Using up to 2 μg/well of sample DNA, increase the number of N so as to make detection possible, and use cases where 1/N is positive to judge positives.

No.	Category 1	Category 2	Question	Answer
Reagent control				
2-15	Operation	Measurement	Is it acceptable to reverse the order of samples to be added to a strip?	If you select a strip that suits your equipment, you will obtain the correct results if you add the samples in the order from A to B or from B to A. Please select the method that is suitable for your PCR system.
2-17	Operation	Detection	What is the maximum amount of DNA in μg per reaction?	Investigations at our company have confirmed that there are no hindrances of PCR reactions up to 2μg/reaction.
2-21	Operation	Detection	How much should be put in for positive control during PCR reactions?	The concentration of positive control of this kit is 2 x 10^3 copies/μL. 1 μL is added to 24μL of DW or a positive sample. When diluting before use, please dilute using 10ng/ul of MS2 RNA (from Roche), Easy dilution (from takarabio), or the like, and do not dilute using a TE buffer or water.
2-22	Operation	Detection	Is ROX dye for passive reference included in the reagent? Also, can ROX dye for passive reference be used?	It is not included in this product. Also, ROX channels are used for positive control, so please do not use Rox dye for passive reference.
			What is the degree of cleanliness of the environments in which inspections are conducted? (Storage and control of reagents, nucleic acid extraction, detection of amplification products, etc.)	It is ideal to separate each of the rooms and perform reagent control and nucleic acid extraction using a clean bench. If this is difficult, one way to do it is to perform reagent control and nucleic acid extraction using separate clean benches or the like. However, performing reagent control and nucleic acid extraction on a laboratory table means that the nucleic acids might spread to the air as aerosol. Therefore, avoiding it will reduce risks.
Detection				
2-18	Operation	Detection	We added the positive control to the cells and evaluated the extraction and detection steps, but PCR did not occur. What is the cause of this?	(1) Extraction reagent that has mixed in to eluted nucleic acid solution may hinder PCR reactions. Before performing elution, thoroughly check that there is no residue of the reagent inside the column. If there is residue, please remove the residue from the vacuum centrifuge and pipette.
2-19	Operation	Detection		(2) If the amount of DNA put into the PCR is too high, it might hinder PCR reactions. Please decrease the amount of DNA and perform PCR.

No.	Category 1	Category 2	Question	Answer
2-20	Operation	Detection		(3) Please recheck your PCR program and florescence detection settings. Also, if the scale of the Y-axis of the amplification curve in the analysis screen is too large, it might not be possible to visually confirm the amplification curve around the area of the lower limit of detection. Please change the scale of the Y-axis as appropriate.
			Is it possible to determine if the strain of mycoplasma is viable bacteria or killed bacteria?	The kit detects mycoplasma genomic DNA, so it cannot determine if the strain is a viable bacteria or killed bacteria.
			Negative control changes to positive.	False positives can result due to contamination. Sources of contamination can exist in various places. (1) The extraction reagent is contaminated. (2) The tip or tube is contaminated, (3) The culture substrate is contaminated, (4) There is contamination during reagent control. In the case of (1) and (4), contamination can result due to positive samples making contact with chips and entering reagents as a result of misoperation. Therefore, in the event that (1) is likely, we recommend performing extraction again using a new reagent. In the case of (2), there are cases where contamination occurs during operation and cases where contamination occurs when a PCR tube, strip, or plate after PCR is put into an autoclave, the autoclave gets contaminated, and then unused tips and tubes get contaminated when put into the autoclave. Therefore, we recommend using tubes, strips, and plates after PCR has been performed or tips and tubes that do not require an autoclave. In the case of (3), there may be contamination of mycoplasma DNA in the raw materials of the culture medium, such as FBS. Therefore, it is necessary check the culture substrate to be used ahead of time, and to select materials in which no contaminants are mixed.
			We want to check positive PCR products using electrophoresis.	There is a high risk of contamination in opening PCR products, so you should avoid opening them. Visual confirmation is not possible in the case of a low concentration (10 to 100 copies), but it is possible if there are at least around 10^5 copies. Reference 1: Pharm tech japan vol32, No.1 (2016) p93