

Implementation of a Microbiological Surveillance Protocol in a Portuguese Tertiary Care Academic Endoscopy Unit

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Keywords

Microbiological surveillance · Protocol · Endoscopy · Endoscope reprocessing

Abstract

Introduction: International societies recommend microbiological surveillance of endoscopes to reduce the incidence of endoscope-associated infections, particularly for duodenoscopes. However, surveillance protocols are not internationally standardized, both regarding sample collection, processing, and culture. This study aims to provide a framework protocol encompassing the experience of a tertiary large volume endoscopy center and the microbiology laboratory for collecting and culturing of endoscope samples for microbiological surveillance. **Methods:** A sample collection and processing protocol was designed as a result of a cooperation between the Endoscopy Center of the Gastroenterology Department and the Microbiology Laboratory of the Department of Clinical Pathology. This protocol reflects international recommendations in this topic and the

human and technological resources of the involved departments. **Results:** The established protocol for collecting samples varies according to the type and model of endoscope. The specimens are collected as sterile saline liquid samples, as well as swabs (with and without transport media). Together with the collection of samples from the endoscope, samples from the final rinse water as well as the water bottle are also collected. For duodenoscopes and curvilinear echoendoscopes, we perform microbiological surveillance every 3 months; for gastroscopes and colonoscopes, at least, once a year; and for specific endoscopes, such as the pediatric or dual-channel therapeutic endoscopes, enteroscopes, or radial echoendoscopes, every 6 months. **Conclusion:** Endoscopy units should have detailed protocols for microbiological surveillance of endoscopes. These protocols should be drawn up by a multidisciplinary team that includes endoscopy nurses, gastroenterologists, microbiologists, and the antimicrobial stewardship team, following international recommendations, adapted to each institution resources.

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Implementação de Protocolo de Vigilância Microbiológica numa Unidade Terciária de Endoscopia Portuguesa

Palavras Chave

Vigilância microscópica · Protocolo · Endoscopia · Reprocessamento endoscópico

Resumo

Introdução: As sociedades científicas internacionais recomendam a vigilância microbiológica para reduzir a incidência de infeções associadas aos endoscópios, particularmente dos duodenoscópios. Contudo, não existe uma padronização internacional dos protocolos de vigilância microbiológica, tanto no que diz respeito à colheita, quanto à cultura e análise das amostras. Este estudo tem como objetivo estabelecer um protocolo com base na experiência de um centro terciário de endoscopia digestiva e do laboratório de microbiologia para a colheita e cultura de amostras de endoscópios para vigilância microbiológica. **Métodos:** Foi elaborado um protocolo de colheita e processamento de amostras como resultado da cooperação entre a Unidade de Endoscopia e o Laboratório de Microbiologia do Departamento de Patologia Clínica de um hospital terciário. Este protocolo reflete as recomendações internacionais nesta área, assim como os recursos humanos e tecnológicos necessários à sua implementação. **Resultados:** O protocolo estabelecido para a colheita de amostras varia de acordo com o tipo e modelo do endoscópio. São colhidas amostras líquidas em meio salino estéril, bem como zaragatoas (com e sem meio de transporte). Simultaneamente, são colhidas amostras da água do enxaguamento final e do copo de água. Para duodenoscópios e ecoendoscópios curvilíneos, foi realizada uma vigilância trimestral; para gastroscópios e colonoscópios, essa vigilância foi realizada, pelo menos, anualmente; para endoscópios específicos, como endoscópios pediátricos ou terapêuticos de duplo canal, enteroscópios ou ecoendoscópios radiais, foi realizada uma vigilância semestral. **Conclusão:** As unidades de endoscopia devem estabelecer protocolos detalhados de vigilância microbiológica dos endoscópios. Estes protocolos deverão ser elaborados por uma equipa multidisciplinar que inclua enfermeiros de endoscopia digestiva, gastroenterologistas, microbiologistas e a equipa responsável pela gestão de antimicrobianos, seguindo as recomendações internacionais, adaptadas aos recursos de cada instituição.

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Introduction

Historically, endoscope-associated infection was deemed to be very rare and the majority of documented cases were believed to be caused by noncompliance with guidelines [1, 2]. Recently, infection risk associated with contaminated, patient-ready flexible endoscopes has been reappraised [3] and several outbreaks of multidrug-resistant organisms have been particularly concerning [1].

A key recommendation is from scientific societies for reducing infection transmission risk focus in the culture of patient-ready endoscopes to detect contamination with organisms of concern [1, 4]. In fact, microbiological surveillance is a tool that we can use to monitor and adapt reprocessing protocols and a quality control measure for this process [2, 5–7], which allows us to identify endoscopes with persistent contamination, despite their reprocessing [8].

Microbiological surveillance can also alert to possible defects in endoscopes and washer-disinfectors, so that endoscopy units can react at an early stage [9]. However, to implement an endoscope microbiological surveillance program, there is a need for specialized human and technical resources, both for sample collection and specimen culture and analysis [8]. Moreover, we must also consider the impact on procedure costs and environmental footprint. All these factors must be taken into account when developing microbiological surveillance protocols in endoscopy units, for them to be feasible in clinical practice.

Protocols for endoscope microbiological surveillance are established in several countries but lack standardization [4, 9]. There are significant variations in the timing and methods of sampling, the number of channels checked, the type of sampling solution used, the microbiological methods used (e.g., filtration vs. centrifugation), and the interpretation of the results [9]. The aim of this report was to summarize the critical aspects of the microbiological surveillance protocol drawn up by the Gastroenterology and the Clinical Pathology Departments in a Portuguese tertiary care academic Hospital.

Methods/Design

Microbiological Surveillance Protocol

The protocol resulted from a multidisciplinary partnership taking into account current scientific societies guidelines and the existing technical and human resources available in our hospital. Decisions were made regarding the frequency, sampling and

Table 1. Microbiological surveillance request template

Microbiological surveillance				
Endoscope identification	Model: Serial number:	Harvest date: ____/____/____ Harvest time: ____:____		
Responsible for the harvest Research to be carried out	• Bacteriological examination • Legionella research • Mycobacteria research			
Identification of samples to be sent	Liquid samples 1. Final rinse water from the AER (100 mL) AER identification: 2. 2A. Water container (100 mL) 2B. Lid tube (100 mL) 3. Suction, air/water, and working channel (100 mL) 4. Elevator channel (5 mL) 5. Auxiliary water channel (5 mL) Swabs Z1. Swab in transport media to suction cylinder, air/water cylinder, and working channel Z2. Swab in transport media to elevator mechanism/recess Z3. Swab without transport media to suction cylinder, air/water cylinder, and working channel Z4. Swab without transport media to elevator mechanism/recess	Labeling ESN – 1 ESN – 2A ESN – 2B ESN – 3 ESN – 4 ESN – 5 ESN – Z1 ESN – Z2 ESN – Z3 ESN – Z4	Sent	Results
Total samples sent	Liquid samples: _____ Swabs: _____			
AER, automatic endoscope reprocessor; ESN, endoscope serial number.				

Table 2. Samples taken according to type and model of endoscope

	Duodenoscope	Curvilinear echoendoscope	Radial echoendoscope	Pediatric endoscope	Pediatric colonoscope	Therapeutic endoscope	Enteroscope	Gastroscope	Colonoscope
Liquid samples									
Aspiration channel	X	X	X	X	X	X	X	X	X
Air/water channel	X	X	X	X	X	X	X	X	X
Working channel	X	X	X	X	X	X	X	X	X
Elevator channel	X (only in models TJF- Q160 and TJF-145)	X	—	—	—	—	—	—	—
Auxiliary water channel	—	—	—	—	X	X	X (except models GIF- Q180 and GIF-Q165)	X	X
Balloon channel	—	—	—	—	—	—	X (Fujifilm only)	—	—
Final rinse water	X	X	X	X	X	X	X	X	X
Water bottle	X	X	X	X	X	X	X	X	X
Swabs									
Aspiration cylinder	X	X	X	X	X	X	X	X	X
Air/water cylinder	X	X	X	X	X	X	X	X	X
Working channel	X	X	X	X	X	X	X	X	X
Elevator mechanism/ recess	X	X	—	—	—	—	—	—	—
Distal end	—	—	X	X	X	X	X	X	X
Type/model of endoscope in the unit	Olympus (TJF-Q190V; TJF-Q180V; TJF-160; TJF-145)	Olympus (UCT-180); Pentax (EG- 38700UTK)	Olympus (GF- UE 190)	Olympus (GIF- H190 N; GIF- XP160)	Olympus (PCF-H190TL)	Olympus (GIF- 2TH180; GIF- 2T160)	Olympus (SIF- 180), Fujifilm (EN-580T)	Olympus (GIF-EZ1500; GIF-1100; GIF-HQ190; GIF-H190; GIF-Q180; GIF-Q165)	Olympus (CF- EZ1500; CF- HQ1100DL; CF- H190L; CF-Q180AL)

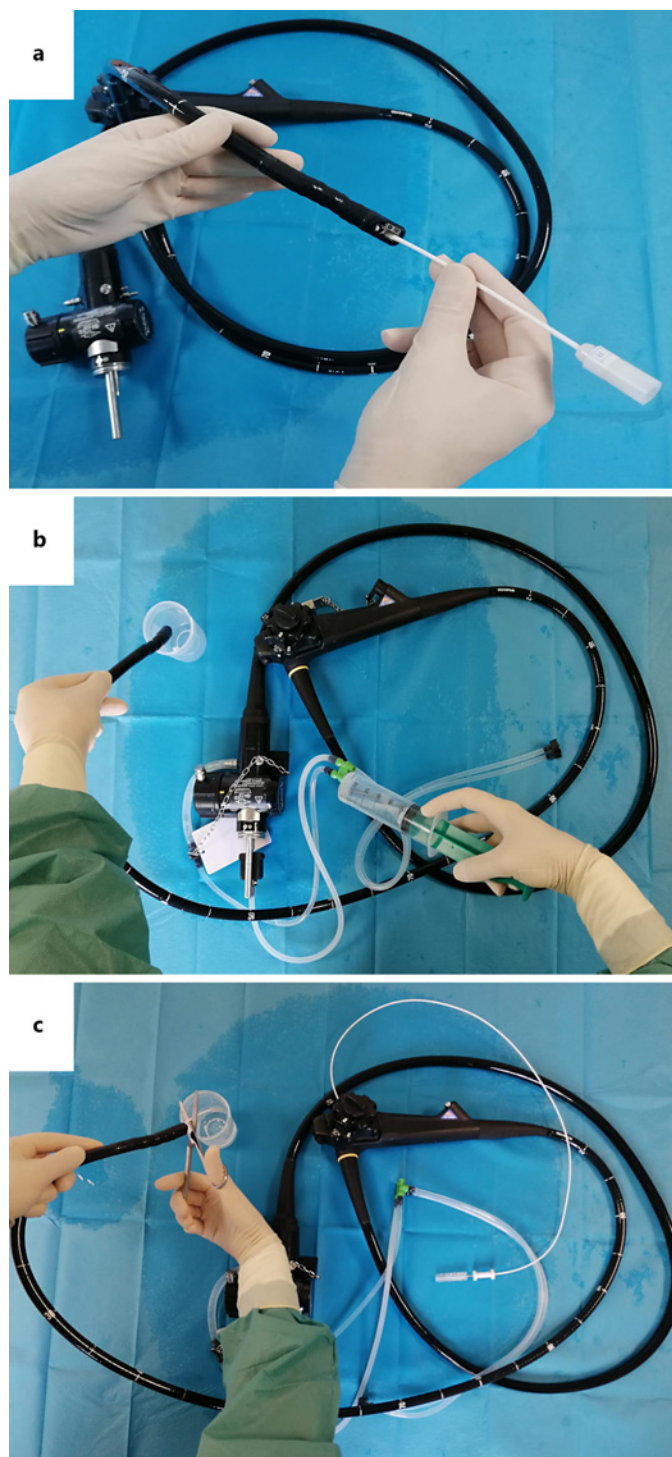


Fig. 1. Collection of microbiological samples. **a** Swab from the elevator recess from duodenoscope/echoendoscope (or from the distal end in the case of endoscopes that do not have an elevator). **b** Liquid sample from the aspiration, air/water, and working channel. **c** “Flush-brush-flush” method.

culture methods and analysis. A microbiology request form was drawn up, accounting for the specificities of these samples (Table 1).

Sample Collection

The sample collection methods depend on endoscope type and model (Table 2). The collected samples are sterile saline liquid samples, as well as swabs (with and without transport media). No neutralizing substances were used, and the samples were transported to the local laboratory within a 30 min timeframe. Liquid samples are taken from the suction, air/water and biopsy channels of the endoscope, regardless the type and model. Then, depending on the presence of other channels, additional samples are obtained, such as from the elevator channel for duodenoscopes and curvilinear echoendoscopes, as well as from the auxiliary water channel in endoscopes that include this channel (colonoscope, gastroscope, pediatric colonoscope, or therapeutic endoscope) and balloon channel in the enteroscope (depending on the endoscope manufacturer). Additionally, samples of the final rinse water and from the water bottle are also collected.

Regarding swabs, samples are collected from the suction and air/water cylinder and biopsy channel of all endoscopes. For endoscopes that include an elevator channel, an additional specimen is taken from the elevator mechanism and from the elevator recess. For other endoscope types, this is replaced by a sample from the external distal end of the endoscope. These samples are collected in duplicate swabs (with and without transport media).

For effective detection of microbial contamination in patient-ready endoscopes, an aseptic technique is used. First, a 100 mL sample of the final rinse water from the automatic endoscope reprocessor (AER) machine is taken. Then, from the water bottle used in the endoscopic procedure, two samples are taken: one directly from the water in the container (100 mL) and the other by irrigating sterile saline through the line of the tube lid (100 mL). These samples are placed in separate containers.

For endoscopes, swabs and liquid samples are collected. Swabs are soaked in sterile saline and only then, the sample is obtained from the suction aspiration cylinder, air/water cylinder, working channel, and elevator mechanism/recess in the case of duodenoscopes and linear echoendoscopes or from the distal end in the case of endoscopes that do not have an elevator mechanism (Fig. 1a). This is done for both swabs with and without transport media. The swab of the suction cylinder, air/water cylinder, and working channel is different from the swab of the elevator mechanism/recess or distal end of the endoscope, so that, in the event of a positive microbiological test, we can identify the microbiological contamination origin. For the liquid samples, 100 mL specimens of sterile saline from the suction channel, air/water channel, and working channel are taken using sterile connectors (Fig. 1b). Halfway through sample collection (after collecting 50 mL), we insert a previously sterilized brush through the suction channel and the working channel and cut the brush to be sent for analysis, together with the liquid sample, in order to improve sensitivity (“flush-brush-flush” method – Fig. 1c). The brush follows a sterilization process with ethylene oxide, as previously described by Ji et al. [10]. This sample is placed in a sterile container.

Table 3. Microbiological timetable surveillance

Microbiological frequency surveillance			
type/endoscope model	every 3 months	every 6 months	annually
Duodenoscope	X	—	—
Curvilinear echoendoscope	X	—	—
Radial echoendoscope	—	X	—
Pediatric endoscope	—	X	—
Pediatric colonoscope	—	X	—
Therapeutic endoscope	—	X	—
Enteroscope	—	X	—
Gastroscope	—	—	X
Colonoscope	—	—	X

In the case of duodenoscopes or echoendoscopes with an elevator channel, or endoscopes with an auxiliary water channel, a 5 mL sample of sterile saline is irrigated through these channels, and it is sent for culture. These samples are placed in different containers.

The sample collection protocol for the duodenoscope is reported in the online supplementary material 1 (for all online suppl. material, see <https://doi.org/10.1159/000539455>). For other types of endoscopes, the protocol must be altered depending on the presence of other channels, such as the auxiliary water channel or balloon channel.

Microbiology Specimen Processing

The samples are processed immediately on arrival at the laboratory. In order to improve microbial recovery, samples with an optimum volume of 100 mL, as well as all samples over 5 mL, are previously concentrated by centrifugation for 15 min at 3,000 rpm. Subsequently, the samples are inoculated into a set of culture media suitable for the growth of the microorganisms to be assessed.

For the bacteriological test, 0.1 mL of each sample is inoculated using the 3-quadrant streak method onto Blood Agar (BioMérieux, Marcy-l'Étoile, France), and incubated at 35° in a CO₂ atmosphere for 5 days. For the detection of *Legionella* species, 0.1 mL of each sample is inoculated using the 3-quadrant streak method onto Buffered Charcoal Yeast Extract (BCYE; Oxoid, Madrid, Spain) and Glycine Vancomycin Polymyxin Cycloheximide (GVPC; Oxoid, Madrid, Spain) media and incubated at 35° in a CO₂ atmosphere for 10 days. For the recovery of mycobacteria, 0.5 mL are inoculated into Mycobacteria Growth Indicator Tube (MGIT; Becton Dickinson, NJ, USA), with Middlebrook 7H9 broth, and incubated and monitored for 42 days using the BD BACTEC MGIT 960 system (Becton Dickinson, New Jersey, USA).

Bacteriological and *Legionella* testing of swab with transport medium samples is carried out by direct inoculation into the culture media and incubation conditions described above. To improve mycobacteriological recovery, the swabs without transport media for this test are previously embedded in MGIT and incubated at 35°C for 24 h, after which the MGIT is incubated under the conditions described for the liquid samples.

Frequency of Surveillance

The timing and frequency for microbiological surveillance are determined by the type of endoscope (Table 3). Endoscopes that include an elevator channel are sampled every 3 months. For specific endoscopes, such as radial echoendoscopes, pediatric endoscopes/colonoscopes and therapeutic (dual channel) endoscopes, we collect samples every 6 months and, for gastroscopes and colonoscopes, annual surveillance is performed.

Timing of Sample Collection

Due to the personnel's working hours, our protocol includes collecting samples within four to 6 h after reprocessing.

Results Report

As the microbiological control is performed under sterile conditions, any microbial growth is valued. In the presence of microbial growth, most of the species are identified using mass spectrometry on the VITEK® MS automated system (BioMérieux, Marcy-l'Étoile, France), based on MALDI-TOF (Matrix Assisted Laser Ionisation Time-of-Flight) technology. Mycobacteria are identified by molecular biology using GenoType Mycobacterium CM/AS (Hain Lifescience, Nehren, Germany), based on the PCR reaction and detection by DNA-strips reverse hybridization and an enzymatic staining reaction.

Discussion

The aim of microbiological surveillance was to ensure the quality of endoscope reprocessing, to identify deficiencies at an early stage and to provide information about possible risks [9]. The European Society of Gastrointestinal Endoscopy (ESGE) and the European Society of Gastroenterology and Endoscopy Nurses and Associates (ESGENA) provide guidance on how to

perform endoscope microbiological surveillance, namely regarding frequency, sampling, sample volume, and culture, for either manual or automated reprocessing. If reprocessing is carried out automatically, the endoscope must be analyzed at the same time as the AER and the water bottle used in the procedure, in order to identify the source of the infection, in the event of a positive microbiological control [5, 11]. The ESGE/ESGENA guidelines recommend implementing microbiological surveillance on all endoscopes [5], while the US Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), and the American Society for Microbiology (ASM) recommend it only for duodenoscopes [8].

The microbiological surveillance protocols used in different institutions differ in the methods of sampling, number of sampled channels, type and volume of sampling solutions and culture methods [1]. Considering the different guidelines and taking into account the resources available in our institution, we developed a protocol that could be implemented in our hospital, respecting its routines and taking into consideration sustainability issues.

In order to reduce the number of liquid samples from each endoscope, it was decided to combine certain samples in the same container, taking care not to interfere with the interpretation and analysis of the results or the corrective actions to be implemented, in the case of a positive control. Indeed, samples from different locations were combined ensuring that, in case of positive results, the following corrective measures were similar. As such, samples from the suction channel, air/water channel, and working channel were combined in the same container. Samples of additional channels, such as the elevator channel, auxiliary water channel, or balloon channel, are taken into another container, as well as samples from the final rinse water and water bottle in separate containers.

Regarding swabs, we collect samples from the suction cylinder, air/water cylinder, and working channel with the same swab and then use a second swab to sample the elevator mechanism/recess in duodenoscopes and linear echoendoscopes or in the case of other endoscopes, this is replaced by a sample from the distal end of the endoscope. It was decided to replace and not add this sample, in order not to increase the number of swabs needed from each endoscope and, once again, not to invalidate the implementation of the protocol in clinical practice. Therefore, our protocol includes collecting four swabs for each endoscope (two in transport media and two without transport media), in order to meet the laboratory capacities and reduce costs. At our endoscopy unit, we use

sterile saline for irrigation through the water pump. Besides avoiding contamination, the use of sterile saline is intended to maintain an optimal environment for electric conductivity when performing polypectomy/mucosectomy. The saline vials are “single-day use”; therefore, they are discarded at the end of each shift. Thus, we opted to not include samples from these vials in our protocol. Nevertheless, for endoscopy units using reusable vials for water instillation through the water pump it would be relevant to include samples both from the vial as well as from the water itself.

For liquid sample collection, we selected the “flush-brush-flush” method. It is recommended by the CDC and was shown to be a more sensitive sampling technique in a Chinese study published in 2021 when compared with the conventional “flush method” (91.8 vs. 81.6% qualification rate) [8, 10]. No debris was found during sample collection.

With respect to sampling frequency, the recommendations are controversial, particularly concerning duodenoscopes. The ESGE/ESGENA guidelines recommend duodenoscope microbiological surveillance to be performed at least every 3 months, while, e.g., the World Gastroenterology Organization, in its recently published guideline, recommends it to be carried out monthly [5, 12]. A joint position statement by several Italian societies recommends monthly monitoring of duodenoscopes or after every sixty procedures [4].

Regarding frequency, and in order to be able to include all endoscopes in the surveillance program, we have chosen to follow the European recommendations, taking samples every 3 months from duodenoscopes and curvilinear echoendoscopes, and, at least, annually for other endoscope types, with specific endoscopes such as pediatric, therapeutic, enteroscopes, and radial echoendoscopes being sampled twice a year. With this frequency, we can conciliate the microbiology laboratory response with the inclusion of all endoscopes in use at our unit in the surveillance program, with the possibility for repeating sampling on endoscopes with positive results, after the implementation of corrective measures.

Our protocol has some limitations. First, our protocol focuses on the timing for sample collection. The Italian multisociety position paper recommends sampling to be performed at least 6 to 12 h after storage, to increase the likelihood of biofilm growth [4]. However, at our institution, we have only been able to collect samples within four to 6 h after reprocessing, due to the working hours of the departments and the professionals involved in sample collection, culture, and analysis. This fact can significantly impact on culture results, and in future studies we plan to

evaluate this variable also taking into account endoscope storage conditions. With respect to the eluent solution used for sample collection, the fact that we do not use a buffered solution may be pointed as another limitation that can impact microorganism recovery and viability. In order to overcome this limitation, the samples are sent to the laboratory immediately after collection because it is known that delays in sample delivery and culturing can negatively interfere with the results.

In conclusion, endoscopy units should have detailed protocols for microbiological surveillance of their endoscopes as this is considered a structural quality indicator, to ensure high reprocessing quality [7]. These protocols should be drawn up by a multidisciplinary team that includes endoscopy nurses, gastroenterologists, microbiologists, and the antimicrobial stewardship team, guided by scientific society's recommendations but adapted to each institution resources. The endoscopy units must establish a strong partnership with the microbiology laboratory and endoscope manufacturers, so that these protocols can be applied in clinical practice to guarantee patient safety.

Statement of Ethics

Not applicable due to the procedural character of the study, not involving patients.

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Conflict of Interest Statement

The authors have no conflict of interest to disclose.

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Author Contributions

C.M.: protocol design, sample collection, bibliographic review, drafting of the manuscript, and critical revision of the manuscript; J.L.: protocol design, sample collection, bibliographic review, and critical revision of the manuscript; T.R.: drafting of the manuscript and critical revision of the manuscript; M.H.G.: microbiological analyses and reports and critical revision of the manuscript; N.G., L.S., A.C., and C.O.: sample collection and critical revision of the manuscript; F.V.B.: protocol design and critical revision of the manuscript; M.M.R.: protocol design, microbiological analyses and critical revision of the manuscript; G.M.: critical revision of the manuscript; S.B.: protocol design, critical revision of the manuscript, and final approval of the manuscript.

Data Availability Statement

Data will be made available upon reasonable request.