Bacterial contamination rates in extracorporeal photopheresis

Irene Pamler,[1,†] Eva Richter,[1,†] James A. Hutchinson [ID],[2] Viola Hähnel [ID],[1] Ernst Holler,[3] André Gessner,[4] Ralph Burkhardt [ID],[1] and Norbert Ahrens [ID][1,5]

BACKGROUND: Extracorporeal photopheresis (ECP) is an immunosuppressive treatment that involves leukocyte apheresis, psoralen and UV light treatment, and subsequent reinfusion. Patients treated with ECP are usually immunosuppressed. Bacterial contamination therefore poses a much unwanted risk, but incidence data are lacking.

PATIENTS AND METHODS: We screened all 1922 consecutive ECP procedures scheduled within a roughly 3-year period for eligibility. Those with missing data on ECP method (inline or offline) or type of venous access (peripheral or central) were excluded. ECPs with complete aerobic and anaerobic microbial testing of baseline patient blood samples (n = 1637) and of ECP cell concentrates (n = 1814) were included in the analysis.

RESULTS: A test for microbial contamination was positive for 1.82% of the cell concentrates, with central venous access was the most significant risk factor for the contamination (odds ratio = 19). Patient blood samples were positive in 3.85% of cases, but no patients became septic. Staphylococcus spp. were most abundant, and products with bacterial contamination did not cause side effects after reinfusion. There were no significant differences in contamination rates between inline and offline ECP.

CONCLUSION: These findings stress the importance of sterile procedures and the benefits of using peripheral over central venous access for reducing the risk of bacterial contamination in ECP.

Extracorporeal photopheresis (ECP) is a therapeutic procedure consisting of leukocyte apheresis, treatment of the collected cells with 8-methoxypsoralen (8-MOP), ultraviolet A (UVA) light irradiation, that causes covalent binding of 8-MOP with DNA, and subsequent reinfusion of treated product back to the patient without storage.[1] This therapy adds to transplantation tolerance or in autoimmune diseases and is typically applied for patients that are refractory to first line therapy and that are often subject to a complex immunosuppressive regimen.[2]

The required medical devices are available as combined equipment with apheresis and UVA irradiation in a single machine (inline ECP), or as separate devices (offline ECP). The latter requires that the user connects the leukapheresis bag with the UV irradiation bag, as the former is not UVA permeable. The components may be connected prior to the procedure (closed ECP), or during the procedure while the patient is connected (open ECP, sometimes classified differently by regulatory authorities). Possible combinations of these are closed inline, closed offline, and

open offline ECP. Apart from this, both ECP types require connections to apply 8-MOP.

To start with ECP, leukapheresis requires access to peripheral veins or a central venous catheter (CVC). Peripheral venous access used to cause bacterial contamination in 1–2% of healthy blood donors until the advent of pre-donation sampling, a preventive measure that introduced a bag to the tubing system for the initial 15–30 mL of the donation process.[3,4] This in combination with improved skin disinfection lowered the rate of microbial contamination to approximately 0.03%.[5,6] In autologous stem cell transplant (ASCT), on the other hand, bacterial contamination rates of up to 4.5% are still common.[7,8] The affected patients are treated under antibiotic protection, as there is sufficient time between donation and reinfusion to obtain diagnostic test results.[9] In ECP, however, bacterial contamination typically remains undetected, because the ECP product is quickly reinfused without bacterial testing. Undetected bacteria could, in principal, have severe consequences. Up to now, data on bacterial contamination rates in ECP are lacking. Therefore, we retrospectively collected and analyzed data from sterility testing of ECP procedures performed at our institution to get to know the incidence of bacterial contamination in ECP patients and products.

PATIENTS, MATERIALS, AND METHODS

Patients

A total of 1922 ECP procedures scheduled at our hospital from September 26, 2012 through August 31, 2015 were screened for eligibility (Table 1). Most of the patients (79%) had suffered graft-versus-host disease (GvHD) after autologous stem cell transplantation and received treatment on the basis of their clinical symptoms for several weeks to several months. Patients with other conditions like cutaneous autoimmune diseases were usually treated for longer periods. Treatment frequencies were adjusted to clinical needs. Treatments that met eligibility criteria were included in the analysis as outlined in Fig. 1.

ECP treatment protocol

Treatment was carried out as either closed inline or open offline ECP as described above.[10] Closed inline photopheresis can be administered with a single needle and was therefore preferentially but not exclusively used for patients with limited venous access. Other patients received offline ECP.

Photopheresis was carried out as described previously.[10] Briefly, standardized open offline ECP and closed inline ECP procedures were used.[11] Open offline ECP was performed with the Cobe Spectra (Cobe, Terumo BCT), Spectra Optia (Optia, Terumo BCT) or the Amicus (Fresenius Kabi) device using acid citrate dextrose (ACD-A) for anticoagulation. Heparin was additionally used if clotting was observed. Patients received calcium as required. ECP

TABLE 1. Patient characteristics

Patients*	68
Sex (male [%] / female [%])	57,4%/42,6%
Age [years]	53 (2–73)
Diagnoses*	
Acute GvHD	29
Chronic GvHD	25
Sézary's disease	4
Atopy	1
Scleroderma	4
Psoriasis	1
Crohn's disease	1
Bronchiolitis obliterans following lung transplantation	3
Number of treatments per patient	19 (1–136)
Total number of completed treatments	1868
Closed inline ECP (Uvar XTS)	25.3%
Open offline ECP (Cobe)	71.7%
Open offline ECP (Optia)	1.9%
Open offline ECP (Amicus)	0.8%
Missing data on method	0.2%

Numbers represent median value with range in parentheses unless otherwise indicated.
* Total number.

was delivered via a central or peripheral venous access depending on the condition of the patient's veins.

Required connections in open offline ECP were between patient and apheresis tubing set, for sampling and 8-MOP application, to the UV irradiation bag, and back to the patient. Transfusion sets with 200 μm filters were used for the latter.

Closed inline ECP was performed with the Uvar XTS (Therakos). There were no connection steps apart from venous punctuation and sampling with 8-MOP addition.

Sterility testing

Blood samples for sterility testing were taken from the peripheral or central venous line before the start of apheresis. Samples from the cell concentrates were taken before 8-MOP addition and UV illumination at the bedside, as closed inline ECP does not allow removal of the bag. Treatments and sampling were performed in controlled environment equipped with H13 filtered ventilation. Aerobic and anaerobic culture bottles (BD Bactec Standard Anaerobic/F and Aerob/F, respectively) were incubated for 7 days at 30-32°C with a sample volume of 3-5 mL and 7-10 mL, respectively. Positive cultures were isolated and differentiated by matrix-assisted laser desorption ionization-time of flight (MALDI TOF, Bruker Daltonik GmbH Life Sciences) mass spectrometry, and antibiotic resistance testing was performed using the BD Phoenix system.

Statistical analysis

Data was collected in Microsoft Excel 2010, and R was used to calculate statistical significance using the Wilson score interval with continuity correction using its built-in prop.test function.
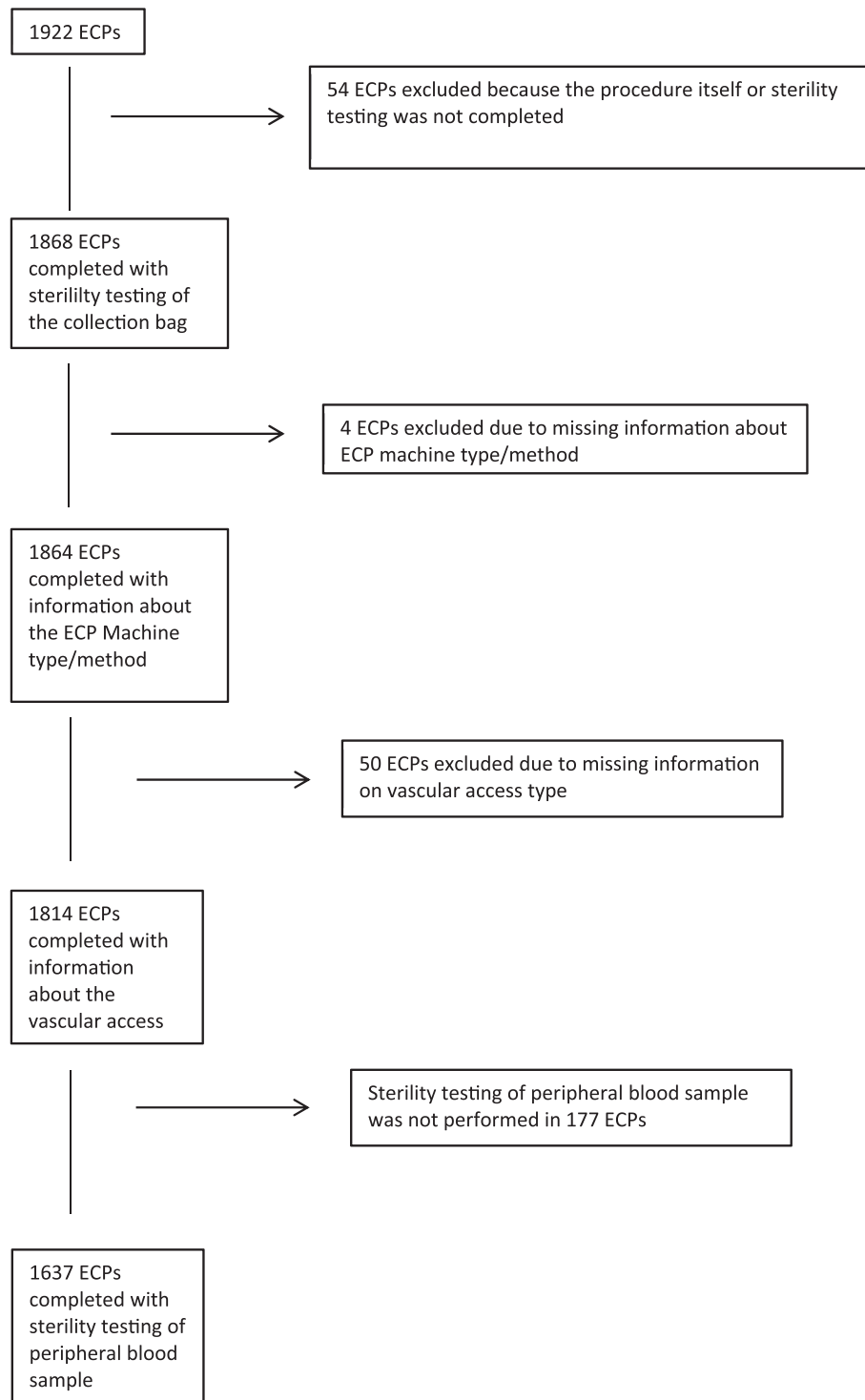
Fig. 1. Patient recruitment and causes of drop out.

RESULTS

Bacterial contamination rate

Complete information on the ECP method (online or off-line), type of vascular access (central or peripheral), and bacterial contamination rate (sterility testing) was available for 1814 ECP cell concentrates. We identified a positive microbial test results in a total of 33 ECP cell concentrates (1.82%). Central venous access, which was required in 20%

of the ECP procedures, was more frequently associated with bacterial contamination of ECP cell concentrates (7.38% positive) than peripheral access (0.41% positive, Table 2, $p < 2.2 \times 10^{-16}$, odds ratio 19). Patient blood samples collected before the start of the procedure were available in 1637 cases. From these, 63 tested positive, with significant differences between central and peripheral venous access (Table 2, p = 0.0026, odds ratio 2.3).

Overall, we found 73 cases of 1637 treatments, in which sterility testing was positive either in cell concentrates and/or patient blood samples. The rate of overlap between procedures with positive ECP cell concentrates and positive patient blood sample was low (16 of 73, 22%). In an additional 10 procedures, only the cell concentrate was positive (14%), and in another 47 instances, only the patient blood sample was positive (64%).

Patient blood samples had a significantly higher contamination rate than samples from cell concentrates (3.85% of 1637 vs. 1.82% of 1814 respectively, p = 0.00044). Contamination proportions in patients' blood and in cell concentrates were not equally distributed. The frequency of positive samples for patients' blood and for cell concentrate samples was unequally distributed (Fig. 2). Patients with fewer ECP treatments tended to have more frequent unsterile findings.

Of note, we did not detect significant differences between inline and offline ECP with regard to bacterial detection rates in ECP cell concentrates or patient blood samples (p = 0.068 and p = 1, respectively, Table 2). In addition, none of the bacterially contaminated products caused adverse events upon reinfusion.

Bacterial species distribution

Samples testing positive for microbiological contamination exhibited a large and heterogenous variety of germs. The majority of the identified bacteria or fungus belong to the human skin flora, mouth flora, and/or intestinal flora: *Staphylococcus epidermidis, Propionibacterium acnes, Acinetobacter Iwoffi, Actinomyces odontolyticus, Staphylococcus capitis, Micrococcus luteus, Staphylococcus aureus, Staphylococcus hominis, Citrobacter freundii, Candida guilliermondii, Enterobacter cloacae, Prevotella bivia, Haemophilus parainfluenzae, Staphylococcus haemolyticus,*

A. Patients with unsterile blood samples
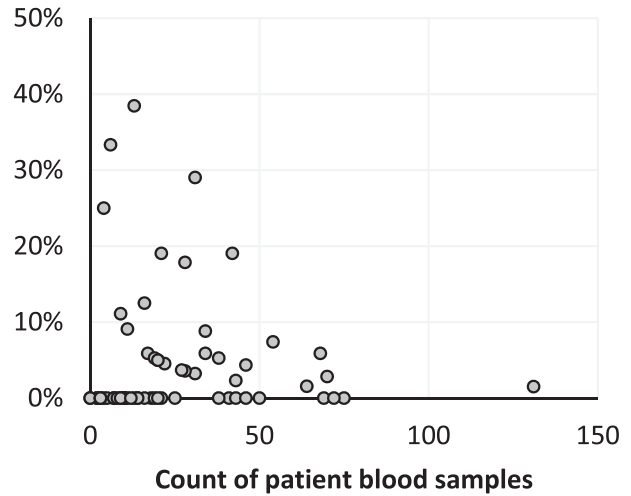
B. Patients with unsterile cell concentrates
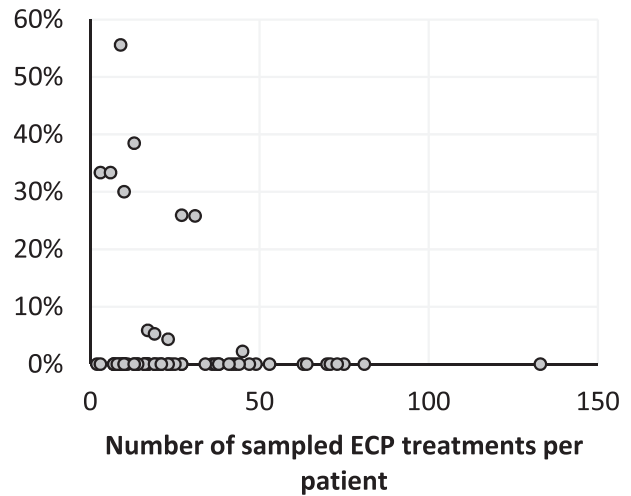
Fig. 2. Proportion of unsterile samples from peripheral or central venous blood (A) or from ECP cell concentrates (B) per patient.

TABLE 2. Microbial detection rates* for (A) offline versus inline ECP and (B) peripheral versus central venous access

	ECP products			Patient blood		
	Percent positive	95% CI	n	Percent positive	95% CI	n
(A)						
Offline ECP	2.18%	(1.50–3.13%)	[30/1378]	3.93%	(2.94–5.22%)	[48/1220]
Inline ECP	0.69%	(0.18–2.17%)	[3/436]	3.60%	(2.10–6.00%)	[15/417]
(B)						
Peripheral access	0.41%	(0.17–0.95%)	[6/1448]	3.11%	(2.27–4.23%)	[41/1319]
Central venous access	7.38%	(5.00–10.68%)	[27/366]	6.92%	(4.49–10.44%)	[22/318]
* Detection rates refer to different sample populations.						

TABLE 3. Bacterial species in ECP cell concentrates and in patients' blood			
	Closed inline ECP*	Open offline ECP*	Patient's blood†
Peripheral venous access			
Staphylococcus epidermidis	–	4	13
Staphylococcus aureus	–	1	13
Prevotella bivia	–	1	1
Propionibacterium acnes	–	–	1
Spore	–	–	1
Acinetobacter Iwoffii	–	–	1
Actinomyces odontolyticus	–	–	1
Staphylococcus capitis	–	–	2
Micrococcus luteus	–	–	2
Staphylococcus hominis	–	–	1
Bacillus altitudinis	–	–	1
Bacillus pumilus	–	–	1
Bacillus subtilis	–	–	1
Citrobacter freundii	–	–	1
Haemophilus parainfluenza	–	–	1
Staphylococcus haemolyticus	–	–	3
Staphylococcus simulans	–	–	1
Staphylococcus lugdunensis	–	–	1
Central venous access			
Staphylococcus epidermidis	3	12	11
Staphylococcus capitis	–	7	5
Pseudomonas aeruginosa	–	1	1
Enterococcus faecium	–	2	–
Klebsiella pneumoniae	–	1	1
Micrococcus luteus	–	–	1
Staphylococcus hominis	–	–	1
Candida guillermondii	–	–	1
Enterobacter cloacae	–	–	1
Capnoctyophaga ochracea	–	1	–

* Data from 1814 procedures.
† Data from 1637 procedures.

Staphyloccous simulans, Staphylococcus lugdunesis, Enterococcus faecium, Klebsiella pneumoniae, and *Capnocytophaga ochracea.*

In addition, some species are environmental bacteria that can be found in soil, air, and/or water: Spores, *Micrococcus luteus, Bacillus pumilus* (resistant to UV light), *Candida guilliermondii, Enterobacter cloacae,* and *Pseudomonas aeruginos.* Interestingly, *Bacillus altitudinis* is a germ which was first isolated from cryogenic tubes used for collecting air samples from high altitudes.[12]

Five patient blood samples contained two different bacteria (*Acinetobacter Iwoffii* and *Staphylococcus aureus, Staphylococcus aureus* and *Staphylococcus epidermidis, Staphylococcus simulans* and *Staphylococcus epidermidis, Staphylococcus lugdunensis* and *Staphylococcus capitis, Prevotella bivia* and *Haemophilus parainfluenza*). In one of these cases, the cell concentrate also tested positive, but only for one bacterial species (*Prevotella bivia*).

The distribution of detected bacterial species separated by ECP system type is shown in Table 3.

DISCUSSION

In this large retrospective study, we found an overall microbial contamination rate of 1.82% in the cell concentrates of extracorporeal photopheresis. This figure is comparable to that of apheresis procedures for the collection of other blood components, such as platelets and hematopoietic stem cells.

Bacterial contamination rates in platelet concentrates from healthy donors reportedly range from 0.01 to 0.2%.[13] The frequency of positive culture tests that failed in confirmation testing is usually higher, i.e., 0.11 to 0.72%.[5,14,15] Negative confirmation testing can occur due to inappropriate diagnostic techniques and may reflect contamination with low numbers of bacteria in the inoculum or with bacteria that require special growth conditions. We did not distinguish between these factors and included all positive findings. Thus, the initial positive frequencies are technically closer to those observed in our patients.

Bacterial contamination rates in hematopoietic stem cell apheresis range from 0.2 to 24%, averaging about 3%.[16] Higher frequencies in patients may be caused by additional handling steps during the procedure. These include disinfection as well as the diversion of the first milliliters after venipuncture for predonation sampling which, however, has been shown to substantially decrease the contamination rate.[17] The type of venous access used is another difference between healthy blood donors and patients undergoing apheresis. In our study, a central venous catheter (CVC) was required in 20% of ECP procedures, and CVC use was associated with a 19-fold higher risk for cell concentrate contamination. Though none of the patients became obviously septic at the time of treatment, these findings illustrate the risk of bacteremia in patients with a central venous line.

Microbial contamination risk was not evenly distributed among the patients. We found a subgroup of patients with a high frequency of contaminations up to a maximum of 56% of the cell concentrates. Patients with high contamination rates had a comparable low treatment number, e.g., because of uncontrollable grade IV graft-versus-host disease. In addition, central venous catheter contamination contributed to positive findings. Bacterial contamination in these patients indicates therefore at least in part the severity of the underlying disease.

Bacterial contamination may be impacted by the type of centrifugation. Recently intermittent flow with the Amicus (Fresenius Kabi) was shown to be disadvantageous in platelet apheresis compared to continuous flow apheresis using the Trima (Terumo BCT).[18,19] This disfavors inline ECP that uses intermittent flow technique. On the other hand, the buffy coat layer of bacteria is unknown. Bacteria could distribute freely in plasma, or they could sediment together with red blood cells and platelets. Intermittent flow apheresis using the Latham bowl technique as in Therakos devices, in contrast to the technique used within the Amicus, collects less selected

cell suspensions in general,[10,20] though there are exceptions to this.[21] Inferior collection ability of inline ECP could translate to a reduced bacterial enrichment. In addition, this method processes lower blood volumes, thus further decreasing the possibility of contamination in bacteremic patients.

Higher collection ability of offline ECP methods translates to successful single day treatments.[22] Inline ECP, in contrast, requires treatments on two adjacent days.

In our study, no significant differences were observed between microbial contamination in inline and offline ECP. Patients were not randomized and preferentially treated offline, if a central venous access was available. Central venous lines, in contrast to venous canula, are handled in a sterile way. However, this is more than outweighed by the risk from inapparent catheter infections. The contamination risk was thus increased in offline ECP in our study.

The difference in microbial contamination rates between ECP cell concentrates (1.82%) and patient blood samples (3.85%) might be explained by the fact that the latter samples were collected before apheresis and, thus, had the same effect as predonation sampling. In addition, the apheresis technology itself could have contributed to bacterial depletion to some degree as described above.

We found 24 different bacterial species with Staphylococcus spp. being most abundant. Most of the bacteria we detected were part of human skin flora. These may indicate a contamination by the handling steps. However, these may also indicate that patients for ECP with skin diseases cannot be disinfected successfully with standard procedures. Contamination with the same bacterial type in bag and patient supports this explanation.

The type of bacterial contamination is of relevance from a clinical point of view, as antibiotic susceptibility depends in part on the species. From a technical point of view, there is no contamination that can be regarded as acceptable. There are no commensal bacteria in cell suspensions.

Regardless of the cause of microbial contamination, it is highly unwanted. Patients referred for ECP are usually severely immunosuppressed like in solid organ transplantation or graft-versus-host disease. Their risk for septic complications is therefore increased. On the other side, bacterial contamination of ECP is questionable for two reasons. First, ECP cell concentrates are not stored but reinfused immediately after UV treatment, thus eliminating the chances for bacterial replication. Second, 8-MOP injection with subsequent UV light exposure acts like a pathogen inactivation that reduces bacterial growth potential by several log units.[23] Thus, it can be assumed that any relevant bacterial replication potential is effectively reduced in ECP treatment.

Pathogen inactivation by ECP, however, is speculative. It is therefore mandatory to avoid contamination as much as possible. Disinfection before venipuncture or central line connection was done following a standardized protocol in this study. Patients in need of ECP frequently have skin conditions that are prone to bacterial infections as illustrated by the findings of this study. Thus, effective skin disinfection techniques such as those defined for whole blood donation should also be used in ECP.[24] In addition, the presented data do clearly favor peripheral access for ECP, as contamination by central venous access is by far the most significant risk for bacterial contamination.

This study is the first to evaluate bacterial contamination in ECP cell suspension bags and in ECP patients. Sterility sampling was scheduled for every treatment within the study period, and all patients were considered for inclusion. Most patients and treatments could be included, but dropouts could have impacted the results to some degree. Data have also to be interpreted with caution, as patient and treatment factors with potential impact on contamination, such as venous access and therefore type of ECP, were chosen on a clinical basis and not according to this study. Though it is not possible to conclude on ECP types, it seems reasonable to recognize the contamination risk for ECP in general, especially for patients with central venous access.

The relevance of these findings is unclear, as ECP comprises always pathogen inactivation and cell suspensions would not be stored. This disfavors bacterial testing. The clinical relevance, however, is unclear, and clinical follow up was helpful. In addition, the clinical relevance of inapparently contaminated catheters in these frequently immunosuppressed patients calls for further studies.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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