Agilent Solutions for Removal and Monitoring of Genomic DNA from Monoclonal Antibody Preparations

Application Note

Biotherapeutics & Biosimilars

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Abstract

This Application Note describes an application solution for the removal and monitoring of genomic DNA contamination from monoclonal antibody preparation. The genomic DNA (gDNA) spiked IgG1 was separated using the Agilent 1260 Bio-inert Quaternary LC system and an Agilent Bio-monolith QA column. An analytical-scale fraction collector with peak-based fraction collection was employed to collect the fractions for QC check. On this column, IgG1 was found in the flow through (FT) under the chromatographic conditions. An Agilent 2200 TapeStation system using the Genomic DNA ScreenTape assay was used to monitor the DNA removal. Trace amounts of DNA, if any, in the IgG1 (FT) were PCR amplified using the Agilent SureCycler 8800 system and then re-analyzed using the D1000 ScreenTape assay. These easy steps make this solution particularly suitable for Host cell impurity analysis for the Biopharma industry.

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Introduction

Monoclonal antibodies have become the most widely sought biological agent in the field of molecular biology and medical research. With greater understanding of their immuno-modulatory properties, they have become the next generation of therapeutic agents for treatment of human diseases. This demand has led to a rapid increase in the number of monoclonal antibodies entering clinical trials, with more than 25 already approved worldwide[1].

The Food and Drug Administration (FDA) requires testing of recombinant proteins for the presence of residual nucleic acids. The nucleic acids mainly host cell genomic DNA (gDNA) or retroviral RNA, which come from the manufacturing process, and are are considered contaminants. Nucleic acid fragments greater than two kilobases are also considered to be carcinogenic and have to be removed to comply with regulations.

This Application Note demonstrates a workflow solution for removal and monitoring of residual gDNA in monoclonal antibody preparations by spiking gDNA into IgG1. The nucleic acid removal was accomplished using an Agilent Bio-monolith QA column and Bio-inert LC. The QC was carried out using the Agilent 2200 TapeStation and the SureCycler 8800 systems.

Materials and Methods

Reagents kits and instruments

Agilent 2200 TapeStation system (G2964AA), Genomic DNA ScreenTape and Reagents (5067-5365 and 5067-5366), D1000 ScreenTape and Reagents (5067-5582 and 5067-5583) were obtained from Agilent Technologies and used in accordance to manufacture guidelines.

Qiagen DNeasy Blood and Tissue Kit (Qiagen, #69504) were purchased and used as recommended for genomic DNA extraction. Agilent 8800 SureCycler (G8800A) and Herculase II Fusion DNA Polymerase (#600675) were obtained from Agilent Technologies (Santa Clara, U.S). Primers designed using Primer 3 program were synthesised and purchased from Sigma-Aldrich (Bangalore, India). YPD Broth (#Y1375-250G) and Lyticase (#L4025) were purchased from Sigma-Aldrich.

Genomic DNA extraction and spiking

Pichia pastoris culture was inoculated in YPD broth and grown overnight at 37 °C with shaking. The cells were harvested at OD600 of 0.44 (mid log phase) giving an approximate cell density of 6×10^6 cells per mL. The cells were pelleted and stored at –80 °C until further use. Genomic DNA was extracted using the Qiagen DNeasy kit following a modified protocol optimized with an additional enzymatic digestion step using Lyticase[2]. The vacuum concentrated gDNA (500 ng) was reconstituted using IgG1 at a final concentration of 1 mg/mL and used as a spiked sample. The extracted gDNA was assessed for integrity using the Genomic DNA ScreenTape assay. A volume of 3 µL of gDNA ladder was aliquoted into the first tube of an Optical Tube 8x strip. A 1 µL amount each of extracted gDNA, spiked sample, flow through (FT) from LC, IgG1, and blank was aliquoted into the subsequent tubes and was mixed with 10 µL of gDNA sample buffer. The samples were mixed by vortexing, and spun down briefly to collect the contents at the bottom of the tube. Together with a Genomic DNA ScreenTape, the samples were then placed in the 2200 TapeStation system and analyzed.

LC System and Modules

A completely biocompatible Agilent 1260 Infinity Bio-inert Quaternary LC System with a maximum pressure of 600 bar consisting of the following modules was used:

- Agilent 1260 Infinity Bio-inert Quaternary LC Pump (G5611A)
- Agilent 1260 Infinity Bio-inert High Performance Autosampler (G5667A)
- Agilent 1200 Infinity Series Thermostat (G1330B)
- Agilent 1260 Infinity Thermostatted Column Compartment containing bio-inert click-in heating elements (G1316C, option 19)
- Agilent 1260 Infinity Diode Array Detector with 60-mm Max-Light high sensitivity flow cell (G4212B option 33)
- Agilent 1260 Infinity Bio-inert Analytical-scale Fraction Collector (G5664A)
- Agilent Bio-Monolith QA column (p/n 5190-2511)

The complete sample flow path is free of any metal components such that the sample never comes in contact with metal surfaces. Solvent delivery is free of any stainless steel or iron components.

Anion exchange chromatography parameters

Chromatographic parameters for anion exchange chromatography using the Agilent 1260 Bio-inert LC System are shown in Table 1.

PCR analysis

Primers targeting *beta*-tubulin gene was designed using Primer 3 program (Table 2). PCR master mix was prepared and a 11 µL amount was mixed with 9 µL of gDNA giving a load of 200 ng and was used as positive control. The two FT fractions from the anion exchange chromatography were pooled into a single sample and a 9 µL amount was used as the test sample. No template control (NTC) was included as the negative control. The samples were briefly mixed and vortexed to collect the contents at the bottom of the tube. PCR conditions were 2 minutes at 95 °C, then 35 cycles at 95 °C for 20 seconds, 61 °C for 20 seconds, and 72 °C for 30 seconds. A final extension was carried out at 72 °C for 3 minutes, and the amplicons were used in subsequent analysis.

Software

- OpenLAB CDS ChemStation Edition for LC Systems, Rev.C.01.05
- TapeStation Analysis Software A.01.04

Results and Discussion

In biopharmaceutical productions, the principal source of contaminations are cellular contents such as host cell DNA, which require monitored removal to an extremely low level to meet regulatory requirements.

Figure 1 shows the elution profile of IgG1 spiked with gDNA. On this column, IgG1 was found in the FT under the chromatographic conditions and was collected in the A6 well of the micro-titre plate in the fraction collector.

Table 1. Chromatographic parameters.

Parameters	Conditions
Mobile phase A	10 mM *Tris* Buffer, pH 8.8
Mobile phase B	10 mM *Tris* Buffer, pH 8.8 + 1 M NaCl
TCC temperature	Ambient
Isocratic run	0–100 B in 10 minutes
Injection volume	10 µL (1 mg)
Flow rate	0.8 mL/min
UV detection	280 and 260 nm

Table 2. Primer sequence targeting *beta*-tubulin gene of *Pichia pastoris*.

Gene	Primer	Sequence	Tm °C
Beta-tubulin	Forward	GGGTCTCGACATGTCTGCTA	58
	Reverse	GTAGGTGCCACAAGTCCTCT	59

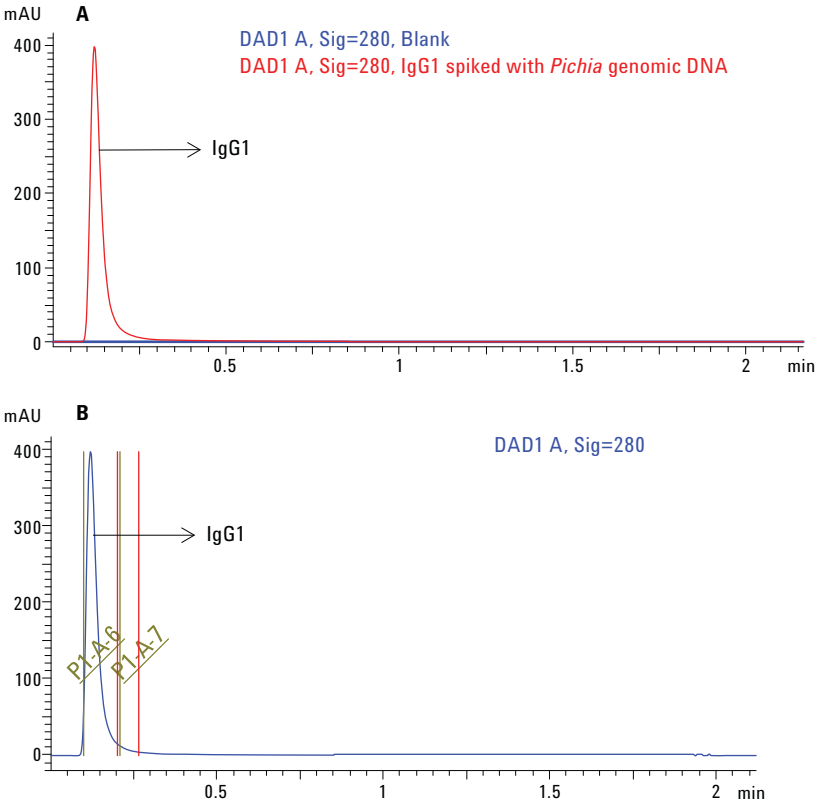

Figure 1. Elution profile of IgG1 spiked with *Pichia* genomic DNA (A). Peak based fraction collection window are marked (B).

Genomic DNA extraction and spiking

The load and the FT from the anion exchange chromatography was analyzed using the Genomic DNA ScreenTape assay along with a buffer (blank) and IgG1 as controls. The gel image in Figure 2A shows the extracted gDNA (B1), IgG1 sample spiked with gDNA (C1), FT fraction (D1), blank (E1), and IgG1(F1) analyzed using the Genomic DNA ScreenTape assay. The image shows a single intact peak of the gDNA in lanes B1 and C1. The difference in signal intensity corresponds to the concentrated gDNA used in spiking IgG1 (C1). The FT from anion exchange chromatography shows absence of gDNA, indicating nucleic acid adsorption to the monolith column. The samples in lanes E1 and F1 show that there is no background signal from the blank and IgG1 on the Genomic DNA ScreenTape assay.

The electropherogram trace in Figure 2B shows the overlays of the spiked IgG1, FT, and blank, indicating the absence of gDNA in the FT fraction.

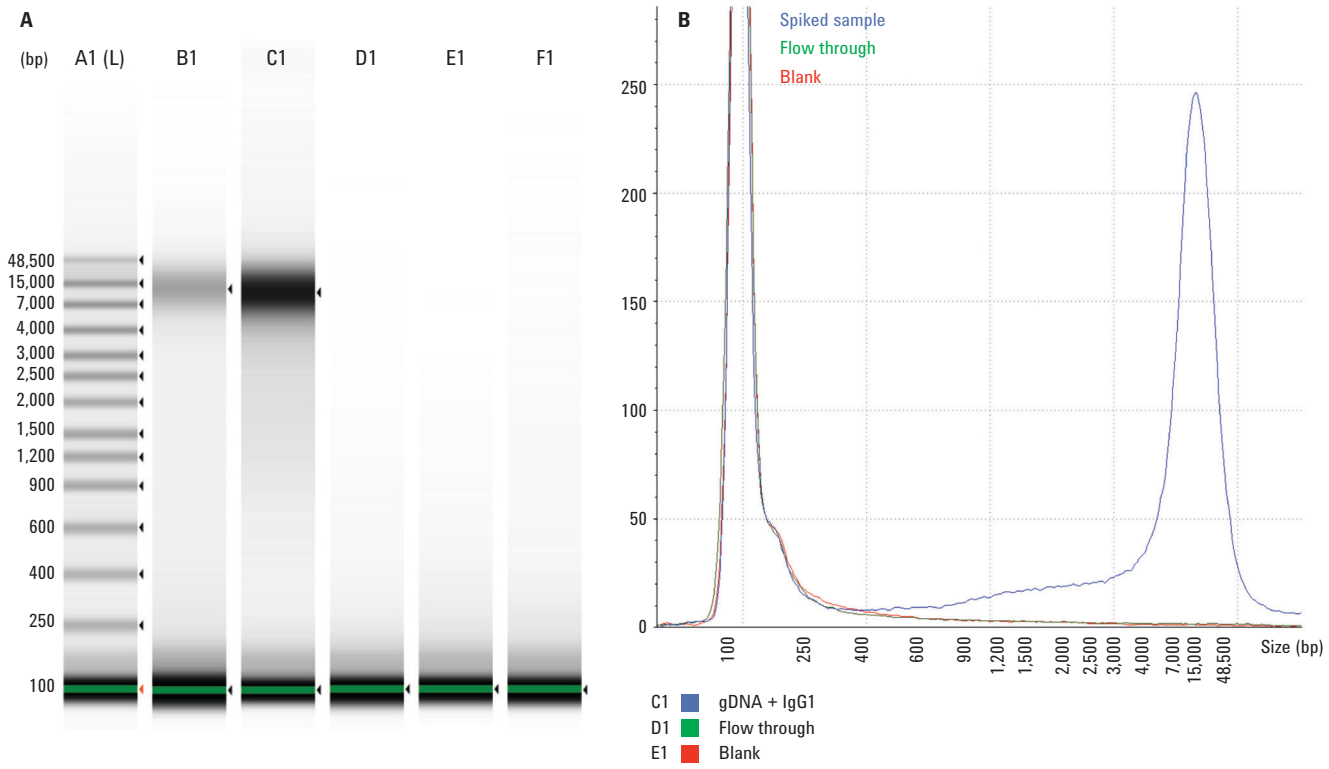

Figure 2. TapeStation gel image showing the gDNA, spiked IgG1, FT, and controls (A). Electropherogram overlay of spiked sample, FT, and blank (B).

PCR analysis of the FT

The amplicons from the PCR reaction were analyzed using the D1000 ScreenTape assay. The gel image in Figure 3 shows the presence of PCR products in positive control and the load, but no amplification products in the FT and NTC. The analysis confirms the absence of DNA in the FT, indicating the removal of nucleic acid contaminant.

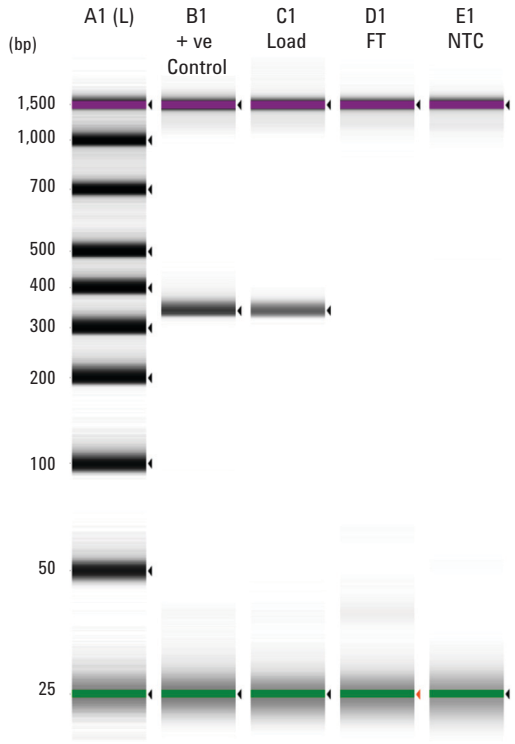

Figure 3. TapeStation gel image showing PCR amplicons from positive control, load, FT, and NTC.

Conclusion

Biopharmaceutical manufacturers must ensure that their products are free from host impurities such as nucleic acids, host cell proteins, endotoxins, viral particles, and process intermediates. Anion exchange chromatography is the method of choice for DNA removal. This Application Note presents several Agilent tools for DNA removal and monitoring. We first used the Agilent 1260 Infinity Bio-inert Quarternary LC system and Bio-monolith QA column for DNA impurity removal with spiking experiments. Additionally, the Agilent 2200 TapeStation and Agilent SureCycler 8800 systems provide certainty regarding the efficiency of the DNA removal. This workflow solution is ideal for DNA impurity removal and monitoring for biologics.

References

1. J.M. Reichert "Antibodies to watch in 2010" *mAbs* 2, 1–16 (**2010**).

2. Purification of total DNA from yeast using the DNeasy Blood & Tissue Kit - (EN) (DY13 Aug-**06**), Qiagen. http://www.qiagen.com/resources/download.aspx?id=1c03b02a-1ddd-4b59-94ec-c0608de2f708&lang=EN&ver=3

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