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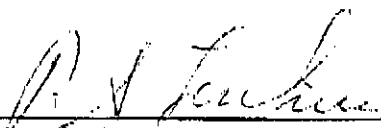
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10 JULY 1997

REPORT ON  
MITOMYCIN RESIDUES  
FOLLOWING DECONTAMINATION  
OF SUPERGEN FACILITY

Submitted by:

  
\_\_\_\_\_  
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Director, Product Development  
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*Report on Mitomycin Residues Following Decontamination of SuperGen Facility***SUMMARY**

Based on the measured limit of determination for Mitomycin in the wipes, all seventeen-tested 12cm wipe samples (floor) showed less than .2 $\mu$ g Mitomycin. The three 15cm wipe samples (2 ceiling, 1 floor) were found to have less than .3 $\mu$ g Mitomycin.

**INTRODUCTION**

This report describes the development of the analytical method and its application to test samples of wipes used to detect any residue of Mitomycin (hence referred to as MMC) in a facility occupied by SuperGen. The wipes were applied to various surfaces as described in this report. The wiping process and decontamination were performed by DECON Environmental Services Services Inc. of Hayward, California.

The wipes were extracted with a known volume of deionized water and any extracted Mitomycin was measured by high pressure liquid chromatography (HPLC).

The analytical procedure developed and its limit of determination are described.

**EXPERIMENTAL****A. Limit of Determination /Accuracy**

An accurate volume of a known concentration of MMC was added to a dry blank wipe which was placed in a screw capped culture tube to which was added a sufficient volume of deionized water for a final volume of 5.0 mL. The extraction was performed by repeatedly inverting the tube by hand for 5 minutes or using a Fisher Roto-Rack for 5 minutes. The supernatant water in each tube was analyzed by HPLC for Mitomycin concentration at the following HPLC conditions:

Column: Dipheny reverse phase, 5 micron, 4.0mm I.D.,  
30cm length at 28-35 $^{\circ}$ C  
Mobile Phase: 250mL methanol, HPLC grade  
5 mL 0.83N acetic acid  
1.54 g. ammonium acetate, HPLC grade  
q.s. HPLC grade water to 1000mL  
If HPLC grade materials are used, there is no need to filter through  
a 0.45 $\mu$  filter.  
Flow Rate: 1.0 +/- 0.1 mL/min.  
Injection Volume: 5 microliters  
Detector Wavelength: 365nm at 1.0AUFS  
Temperature: 30-35 $^{\circ}$ C  
Peak Measurement: Peak height

The accuracy of the method as determined by the recovery of known amount of MMC is shown by the data in Table I.

*Report on Mitomycin Residues Following Decontamination of SuperGen Facility***TABLE I****SPIKING OF BLANK (DRY) WIPES  
WITH MITOMYCIN AND RECOVERY RATES**

Wipe Diameter	$\mu\text{g}$ MMC Added <sup>(1)</sup>	Theoretical MMC in Extract $\mu\text{g}/\text{mL}$	$\mu\text{g}/\text{mL}$ MMC Found in Extract	% Recovered
11cm	15 $\mu\text{g}$	3.0 $\mu\text{g}/\text{mL}$	3.57 $\mu\text{g}/\text{mL}$	119
11cm	7.5 $\mu\text{g}$	1.5 $\mu\text{g}/\text{mL}$	1.37 $\mu\text{g}/\text{mL}$	91
11cm	1.5 $\mu\text{g}$	0.3 $\mu\text{g}/\text{mL}$	0.3 $\mu\text{g}/\text{mL}$	100
12cm	7.5 $\mu\text{g}$	1.5 $\mu\text{g}/\text{mL}$	1.28 $\mu\text{g}/\text{mL}$	85

Reference: Lab book, pp. 45-49, 54

(1) The MMC was added using an appropriate volume of a 15 $\mu\text{g}/\text{mL}$  MMC aqueous solution.**B. Limit of Determination**

The lowest concentration of MMC in the extract which can be reproducibly measured on separate days is used for calculating the limit of determination of MMC on a wipe. The limit differed for some wipes due to the larger volume of water needed to extract a larger size wipe. The lowest concentration was experimentally measured by diluting a known concentration of MMC in water and measuring the signal obtained on the chromatograph. This value was found to be 0.03 $\mu\text{g}/\text{mL}$ . The chromatographic response obtained using the HPLC conditions described above is shown in Figure 1. To calculate the limit of determination for each wipe, the volume of water used to extract each wipe is multiplied by the lowest concentration measurable.

**C. Analysis of Wipes Used to Contact Decontaminated Surfaces**

Upon receipt at Custom Testing & Development, the samples were placed in a freezer. The wipes are described as round laboratory filter papers of various sizes. Samples were removed from the freezer and each wipe weighed to determine the amount of water on the wetted test samples. The volume of water was calculated for each wipe by subtracting the weight of a dry wipe of the same size as the wet wipe from the weight of the wet wipe. The difference, in grams, was taken as the volume in mL on each wipe. The wipes were placed in a screw cap culture tube. A sufficient volume of water was added to each test sample to bring the total volume in contact with each wipe as shown in Table II under "Extraction Volume." The tubes were shaken by hand for 5 minutes or placed on a Fisher Roto-Rack for 5 minutes. Each sample was chromatographed within one hour of addition of water used for extraction. Chromatograms of each sample reported in Table II are labelled as Figures 2 through 23.

*Report on Mitomycin Residues Following Decontamination of SuperGen Facility*

D. Discussion:

The chromatographic conditions and extraction procedure demonstrates the ability of the method to accurately recover Mitomycin from the test wipes as shown in Table I.

The sensitivity of the method, 0.2 µg MMC for 12cm diameter wipes and 0.3µg MMC for 15cm diameter wipes has been calculated based on the ability to measure the concentration in the extraction volume shown in Figure I. These values are lower than those reported in the summary report dated 19 June 1997. Those values were based on a quick conservative evaluation whereas the values reported in this report can be confirmed by a more rigorous review of the experimental method and data analysis.

The data obtained on all test wipes reported in Table II indicate no detectable levels of MMC on any of the test wipes examined. The limit of determination, also referred to as the sensitivity of the method, is reported as 0.2µg MMC for 12cm diameter wipes and 0.3µg MMC for 15cm diameter wipes.

The significance of the levels found and the level of detection as it applies to equipment and facility cleaning in a manufacturing facility is discussed in an articles by K.M. Jenkins and A. J. Vanderwielen entitled "Cleaning Validation: An Overall Perspective," in *Pharmaceutical Technology* p 60-73, (1994).

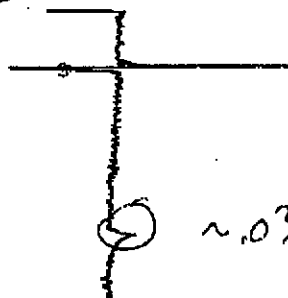
## Report on Mitomycin Residues Following Decontamination of SuperGen Facility

TABLE II

**MITOMYCIN CONTENT OF WIPES USED ON VARIOUS SURFACES  
AFTER DECONTAMINATION OF SUPERGEN FACILITY**

Sample #	Wipe diameter	Location	Extraction volume	$\mu\text{g/wipe}$
Ceiling 1	15cm	Ceiling	9.0mL	<0.3 $\mu\text{g}$
Ceiling 2	15cm	Ceiling	9.0mL	<0.3 $\mu\text{g}$
SG-3	12cm	Floor	6.0mL	<0.2 $\mu\text{g}$
Floor 1	15cm	Floor	9.0mL	<0.3 $\mu\text{g}$
SG-5	12cm	N-Wall	6.0mL	<0.2 $\mu\text{g}$
SG-6	12cm	N-Wall	6.0mL	<0.2 $\mu\text{g}$
SG-7	12cm	S-Wall	6.0mL	<0.2 $\mu\text{g}$
SG-8	12cm	S-Wall	6.0mL	<0.2 $\mu\text{g}$
SG-9	12cm	E-Wall	6.0mL	<0.2 $\mu\text{g}$
SG-10	12cm	E-Wall	6.0mL	<0.2 $\mu\text{g}$
SG-11	12cm	W-Wall	6.0mL	<0.2 $\mu\text{g}$
SG-12	12cm	W-Wall	6.0mL	<0.2 $\mu\text{g}$
SG-13	12cm	Countertop	6.0mL	<0.2 $\mu\text{g}$
SG-14	12cm	Countertop	6.0mL	<0.2 $\mu\text{g}$
SG-15	12cm	Inside L-Cabinet	6.0mL	<0.2 $\mu\text{g}$
SG-16	12cm	Wet Blsnc	6.0mL	<0.2 $\mu\text{g}$
SG-17	12cm	Wet Blank	6.0mL	<0.2 $\mu\text{g}$
SG-18	12cm	Wet Blank	6.0mL	<0.2 $\mu\text{g}$
SG-19	12cm	Wet Blank	6.0mL	Sample lost
SG-20	12cm	Dry Blank	6.0mL	<0.2 $\mu\text{g}$
SG-21	12cm	Dry Blank	6.0mL	<0.2 $\mu\text{g}$
SG-22	12cm	Dry Blank	6.0mL	not tested
SG-23	12cm	Dry Blank	6.0mL	Used for recovery study

START  
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~.03ug/ml

30PPb

FIGURE 1  
Response of Lowest  
Concentration of MMC  
measurable (0.03ug/mL)

CHROMATOPAC C-R3A FILE 1  
SAMPLE NO 0 METHOD 41  
REPORT NO 3495

PKNO	TIME	AREA	NK	IDNO	CONC	NAME
1	3.	10824			100	
TOTAL		10824			100	

START  
97/06/17

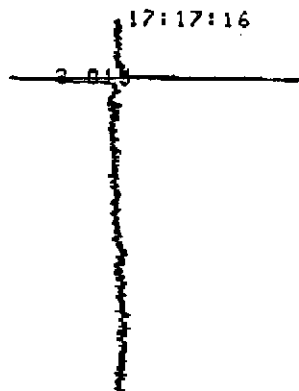


FIGURE 2  
Ceiling 1  
Wipe Extract

CHROMATOPAC C-R3A FILE 1  
SAMPLE NO 0 METHOD 41  
REPORT NO 3541

PKNO	TIME	AREA	NK	IDNO	CONC	NAME
1	3.015	11120			100	
TOTAL		11120			100	

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97/06/17

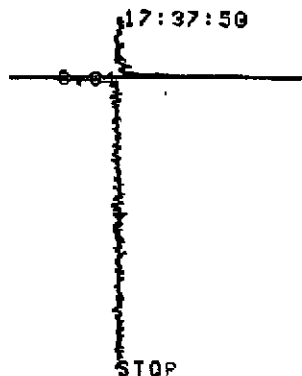


FIGURE 3  
Ceiling 2  
Wipe Extract

CHROMATOPAC C-R3A FILE 1  
SAMPLE NO 0 METHOD 41  
REPORT NO 3542

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97/06/12

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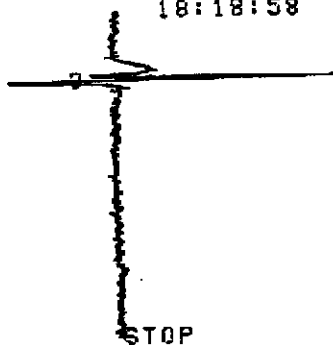


FIGURE 4  
SG-3 Floor  
Wipe Extract

CHROMATOPAC C-R3A  
SAMPLE NO 0  
REPORT NO 3478

FILE 1  
METHOD 41

PKNO	TIME	AREA	NK	IDNO	CONC	NAME
1	3	14586			100	
TOTAL		14586			100	

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97/06/17

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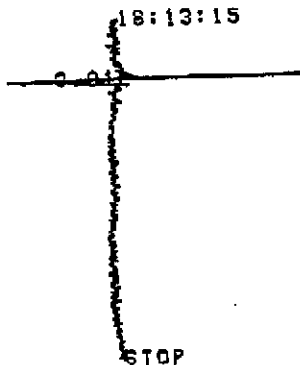


FIGURE 5  
Floor 1  
Wipe Extract

CHROMATOPAC C-R3A  
SAMPLE NO 0  
REPORT NO 3544

FILE 1  
METHOD 41

PKNO	TIME	AREA	NK	IDNO	CONC	NAME
1	3.012	11409			100	
TOTAL		11409			100	

POINT WIDTH(1)  
ANALYSIS PARAMETER FTIF 1

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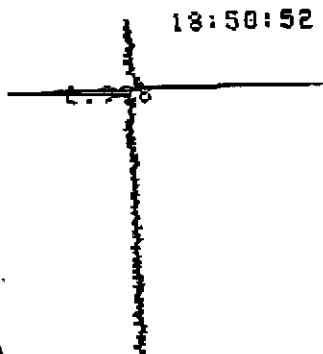


FIGURE 6  
SG-5 N-Wall  
Wipe Extract

7140



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97/06/12

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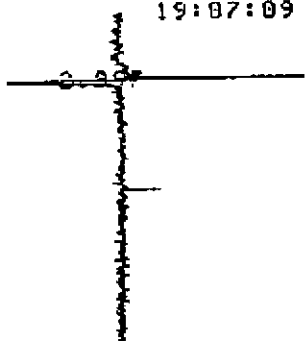


FIGURE 7  
SG-6 N-Wall  
Wipe Extract

CHROMATOPAC C-R3A  
SAMPLE NO 0  
REPORT NO 3481

FILE 1  
METHOD 41

PKNO	TIME	AREA	NK	IDNO	CONC	NAME
1	2.997	9821			100	
TOTAL		9821			100	

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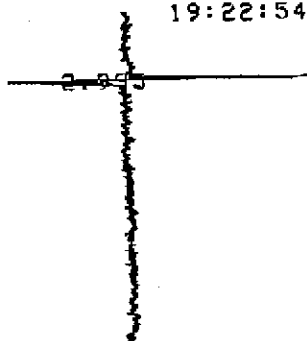


FIGURE 8  
SG-7 S-Wall  
Wipe Extract

CHROMATOPAC C-R3A  
SAMPLE NO 0  
REPORT NO 3482

FILE 1  
METHOD 41

PKNO	TIME	AREA	NK	IDNO	CONC	NAME
1	2.995	10048			100	
TOTAL		10048			100	

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97/06/12

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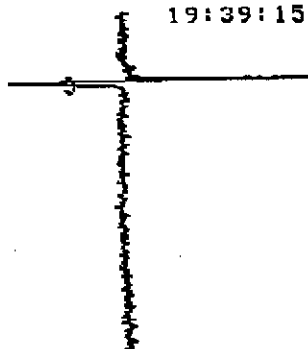


FIGURE 9  
SG-8 S-Wall  
Wipe Extract

CHROMATOPAC C-R3A  
SAMPLE NO 0

3483

FILE 1  
METHOD 41

-----  
 TOTAL 9830 100  
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 97/06/12 19:55:05

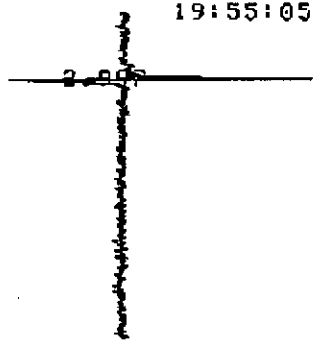


FIGURE 10  
 SG-9 E-Wall  
 Wipe Extract

CHROMATOPAC C-R3A FILE 1  
 SAMPLE NO 0 METHOD 41  
 REPORT NO 3484

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.997	10233			100	
TOTAL		10233			100	

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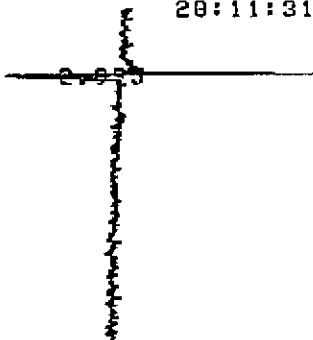


FIGURE 11  
 SG-10 E-Wall  
 Wipe Extract

CHROMATOPAC C-R3A FILE 1  
 SAMPLE NO 0 METHOD 41  
 REPORT NO 3485

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.995	10100			100	
TOTAL		10100			100	

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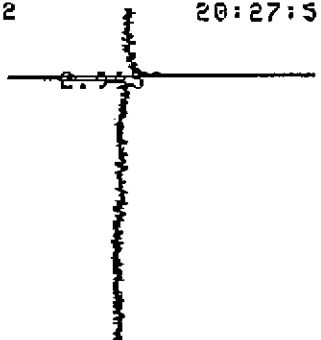


FIGURE 12  
 SG-11 W-Wall  
 Wipe Extract

CHROMATOPAC C-R3A 2486 FILE 1

START  
97/06/12

20:43:46

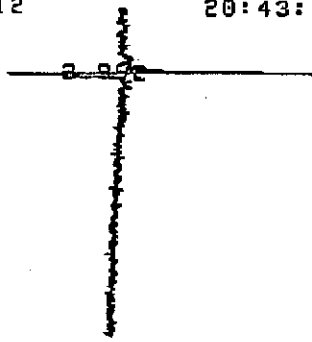


FIGURE 13  
SG-12 W-Wall  
Wipe Extract

CHROMATOPAC C-R3A  
SAMPLE NO 0  
REPORT NO 3487

FILE 1  
METHOD 41

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.992	10278			100	
TOTAL		10278			100	

START  
97/06/12

20:59:39

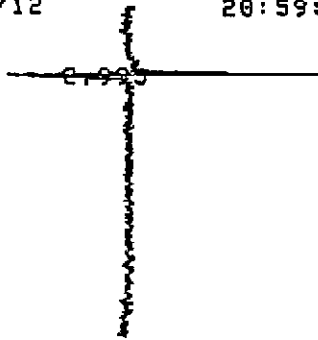


FIGURE 14  
SG-13 Countertop  
Wipe Extract

CHROMATOPAC C-R3A  
SAMPLE NO 0  
REPORT NO 3488

FILE 1  
METHOD 41

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.995	10255			100	
TOTAL		10255			100	

START  
97/06/12

21:15:28

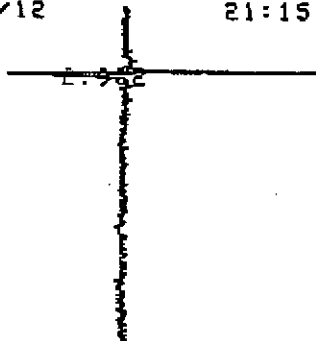


FIGURE 15  
SG-14 Countertop  
Wipe Extract

CHROMATOPAC C-R3A  
SAMPLE NO 0

3489

FILE 1  
METHOD 41

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97/06/12 21:31:17

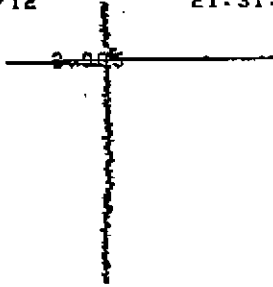


FIGURE 16  
SG-15 Inside L Cabinet  
Wipe Extract

CHROMATOPAC C-R3A  
SAMPLE NO 0  
REPORT NO 3490

FILE 1  
METHOD 41

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	2.985	10547			100	
TOTAL		10547			100	

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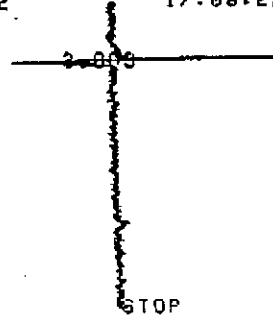


FIGURE 17  
SG-16 Wet Blank  
Wipe Extract

CHROMATOPAC C-R3A  
SAMPLE NO 0  
REPORT NO 3475

FILE 1  
METHOD 41

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.003	10277			100	
TOTAL		10277			100	

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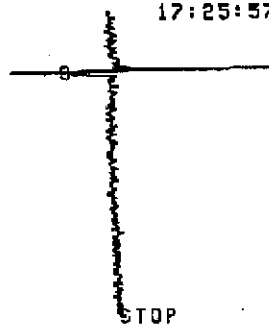


FIGURE 18  
SG-17 Wet Blank  
Wipe Extract

CHROMATOPAC C-R3A  
SAMPLE NO 8  
REPORT NO 3476

FILE 1  
METHOD 41

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3	10291			100	
TOTAL		10291			100	

START

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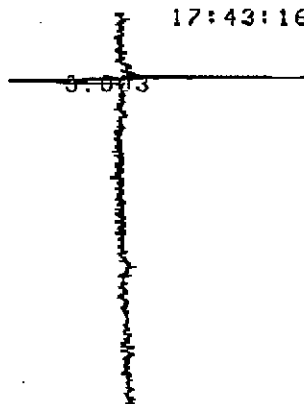


FIGURE 19  
SG-18 Wet Blank  
Wipe Extract

3477

START

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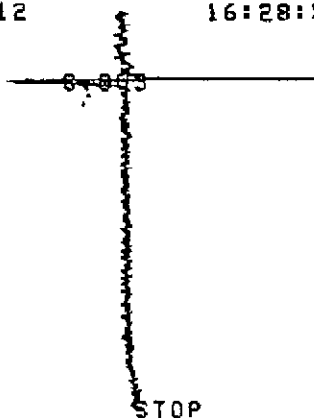


FIGURE 20  
SG-20 Dry Blank  
Wipe Extract

CHROMATOPAC C-R3A  
SAMPLE NO 0  
REPORT NO 3473

FILE 1  
METHOD 41

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.003	10474			100	
TOTAL		10474			100	

START

97/06/12

16:48:07

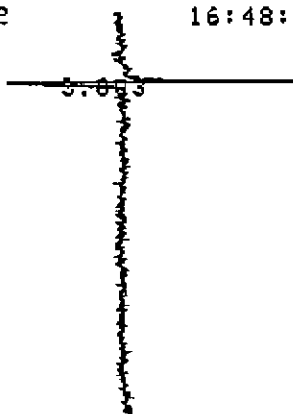


FIGURE 21  
SG-21 Dry Blank  
Wipe Extract

CHROMATOPAC C-R3A  
SAMPLE NO 0  
REPORT NO 3474

FILE 1  
METHOD 41

016