21120.9249 SARS CoV 2 RT qPCR Swab and Isohelix-Saliva Validation Report

Copy of version 3.0 (approved and current)

Last Approval or Periodic Review Completed 05-Oct-2020

Periodic review not required

Effective Date 05-Oct-2020

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Organization ViraCor

Author
Mark Wissel

Comments for version 3.0

Addition of (b) (4) results for an (b) (4) and results for RT-PCR (b) (4) comparison. Updated tables for (b) (4) this upload to correct a typo. This version fixes table 25, which was entered incorrectly.

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	(b) (6)	05-Oct-2020 20:30	3.0	(b) (6) (b) (6)	
Approval	(b) (6) Approver -	05-Oct-2020 11:41	3.0	(b) (6) (b) (6)	
Approval	(b) (6)	29-Aug-2020 9:43	2.0	(b) (6) (b) (6)	

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Approval	(b) (6) Approver -	28-Aug-2020 16:06	2.0	(b) (6)	(b) (6)
Approval	(b) (6)	02-Jun-2020 20:07	1.0	(b) (6)	(b) (6)
Approval	(b) (6) Approver -	02-Jun-2020 16:06	1.0	(b) (6)	(b) (6)

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
3.0	Approved and Current	Major revision	01-Oct-2020	05-Oct-2020	Indefinite
2.0	Retired	Major revision	27-Aug-2020	29-Aug-2020	05-Oct-2020
1.0	Retired	Initial version	01-Jun-2020	02-Jun-2020	29-Aug-2020

Linked Documents

- 21120.9184 SARS CoV 2 RT qPCR Swab and Saliva Validation Protocol

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BioPharma Specific Validation Report to Establish the Performance Characteristics of the SARS Coronavirus 2 (SARS-CoV-2) Reverse Transcription Quantitative Real Time PCR assay in Human Swab and Saliva Specimens

A. Introduction / Objective

An outbreak of coronavirus disease 2019 (COVID-19) caused by the 2019 novel coronavirus (SARS-CoV-2) began in Wuhan, Hubei Province, China in December 2019, and has spread throughout China as well as numerous other countries, including the United States. The outbreak was declared a Public Health Emergency of International Concern on 30 January 2020 by the World Health Organization. Signs and symptoms of COVID-19 include fever, cough, and shortness of breath. Person-to-person spread of SARS-CoV-2 appears to occur mainly by respiratory transmission. How easily the virus is transmitted between persons is currently unclear. Based on the incubation period of illness for Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) coronaviruses, as well as observational data from reports of travel-related COVID-19, CDC estimates that symptoms of COVID-19 occur within 2 – 14 days after exposure. Preliminary data suggest that older adults and persons with underlying health conditions or compromised immune systems might be at greater risk for severe illness from this virus.

The primary objective of this study is to evaluate the ability of SARS-CoV-2 virus (SARS-CoV-2) specific reverse transcription real-time PCR (RT-qPCR) to detect SARS-CoV-2 RNA in swab and saliva specimens. This assay is intended for quantitative detection of RNA from SARS-CoV-2 virus.

This validation report is intended to provide a record of (b) (4) (b) (4) (b) (4) (b) (4) of an in-house developed SARS-CoV-2 assay in human swab and saliva specimens.

B. Scope

This validation report includes the extraction and RT-qPCR method along with assessment of (b) (4) (b) (4) (b) (4) and acceptance criteria for each of these approaches, for the SARS-CoV-2 real-time RT-qPCR assay. **Table 1a** is a summary of the validation data including acceptance criteria; **Table 1b** is a summary of the (b) (4) data.

C. Abbreviations and Definitions

-80°C	-64°C to -90°C, standard storage condition unless otherwise stated
-20°C	-15°C to -35°C
Refrigerated (4°C)	2°C to 8°C
Ambient	15°C to 25°C
37°C	36°C to 38°C
(b) (4)	
mL	milliliter
(b) (4)	
qPCR	Quantitative Real-Time PCR
SD	Standard Deviation
SOP	Standard Operating Procedure

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(b) (4)	(b) (4)
UIC	Universal Internal Control
µL	microliter
(b) (4)	(b) (4)

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Table 1a. Summary of validation criteria and results for SARS-COV-2 RT-qPCR assay in swab and saliva specimens

Performance Characteristic	Action and Acceptance Criteria	Validation Results	Pass/Fail
(b) (4)			

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Table 1a. Summary of validation criteria and results for SARS-COV-2 RT-qPCR assay in swab and saliva specimens

Performance Characteristic	Action and Acceptance Criteria	Validation Results	Pass/Fail
(b) (4)			Pass
			Pass

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Table 1a. Summary of validation criteria and results for SARS-COV-2 RT-qPCR assay in swab and saliva specimens

Performance Characteristic	Action and Acceptance Criteria	Validation Results	Pass/Fail
(b) (4)			Pass
			Pass
			(b) (4)

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Table 1a. Summary of validation criteria and results for SARS-COV-2 RT-qPCR assay in swab and saliva specimens

Performance Characteristic	Action and Acceptance Criteria	Validation Results	Pass/Fail
(b) (4)			
(b) (4)			Pass
(b) (4)			

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Table 1b. Summary of (b) (4) criteria and results for SARS-COV-2 RT qPCR assay in swab and saliva specimens		
Performance Characteristic	**Action and (b) (4) Criteria**	**Results**
(b) (4)		

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Table 1b. Summary of (b) (4) criteria and results for SARS-COV-2 RT qPCR assay in swab and saliva specimens		
Performance Characteristic	**Action and (b) (4) Criteria**	**Results**
(b) (4)		

D. Materials

The following materials (or suitable equivalents) was used:

1. (b) (4)
2. (b) (4)
3. (b) (4)
4. (b) (4)
5. (b) (4)
6. (b) (4)
7. (b) (4)
8. (b) (4)
9. (b) (4)
10. (b) (4) standard Instrument with disposables
11. (b) (4)
12. (b) (4)
13. RNase-, DNase-free water, (b) (4)
14. (b) (4)
(b) (4)
15. Pipette tips with aerosol barrier: 10µL, 200µL, and 1000µL sizes
16. Pipettes to accommodate tip sizes listed above
17. (b) (4) statistical software (b) (4)

E. Methods

Nucleic acid extraction

(b) (4)

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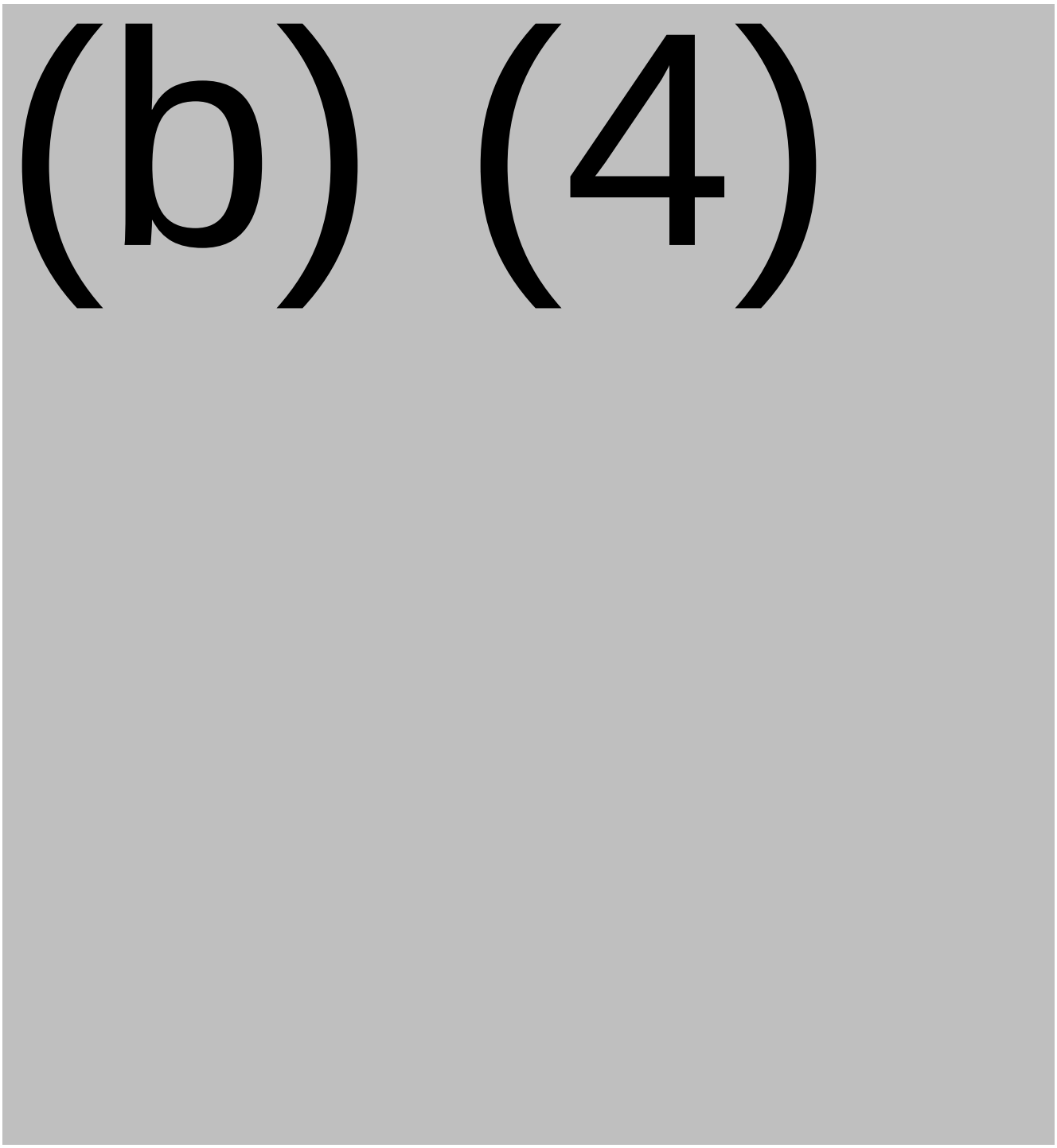
Nucleic acid amplification and detection

Nucleic acid amplification was performed as described in SOP 21120.461 *Real-Time PCR and RT-PCR Using* (b) (4) *Instruments* with the following modifications.

The SARS-CoV-2 RT-qPCR assay is performed as a multiplex reaction with the MS2 internal control assay. Oligonucleotide primers and TaqMan® probes for the detection of the viral N protein gene region of SARS-CoV-2 and an internal extraction and amplification control target (the RNA bacteriophage MS2) were used.



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Acceptance criteria for controls and negative samples:

- (b) (4)

F. Perfo

Matrices to be evaluated and sample creation (**Table 2**)

One type of saliva collection kit made by Isohelix was assessed.

(b) (4)

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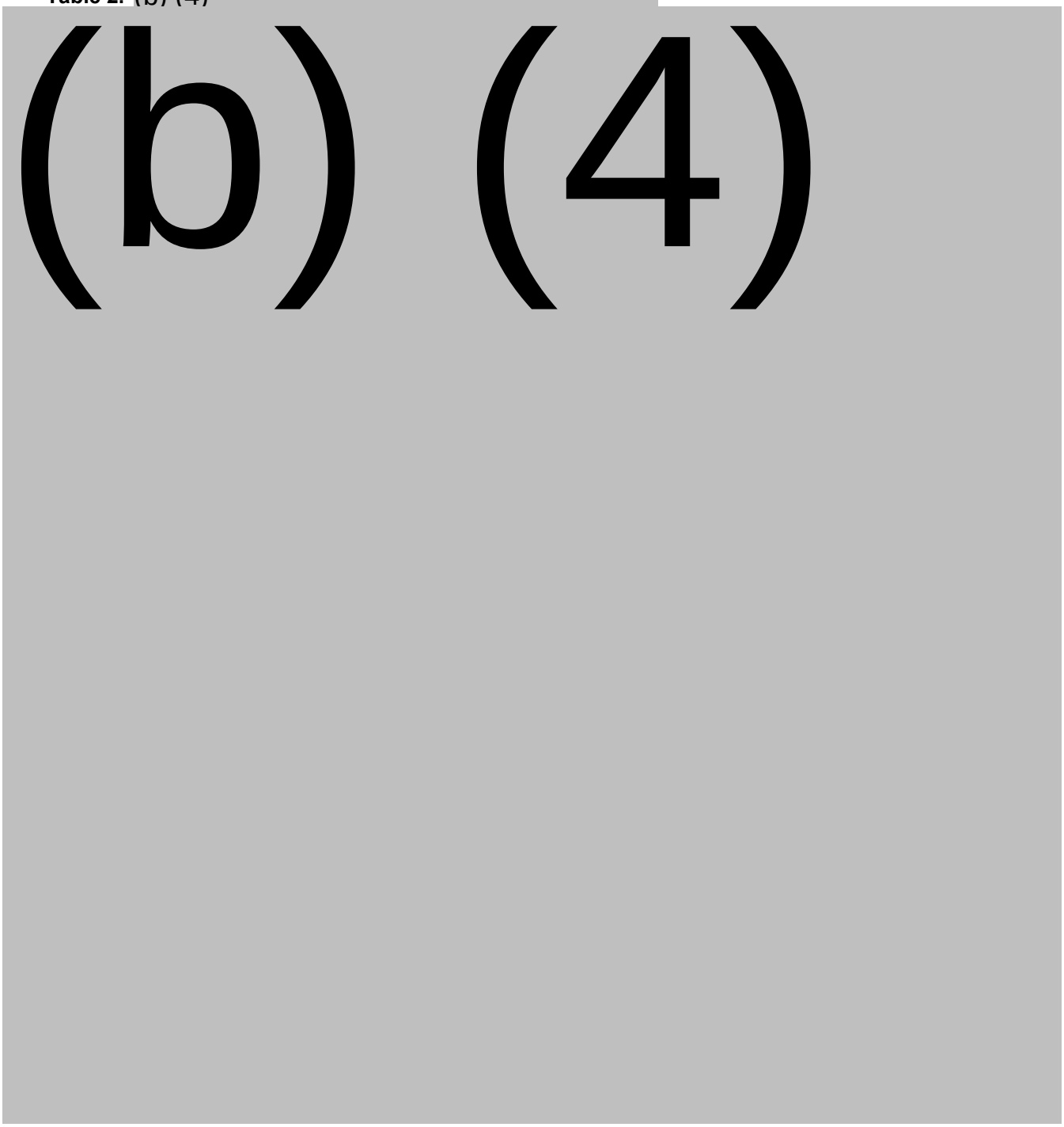
Table 2. (b) (4)



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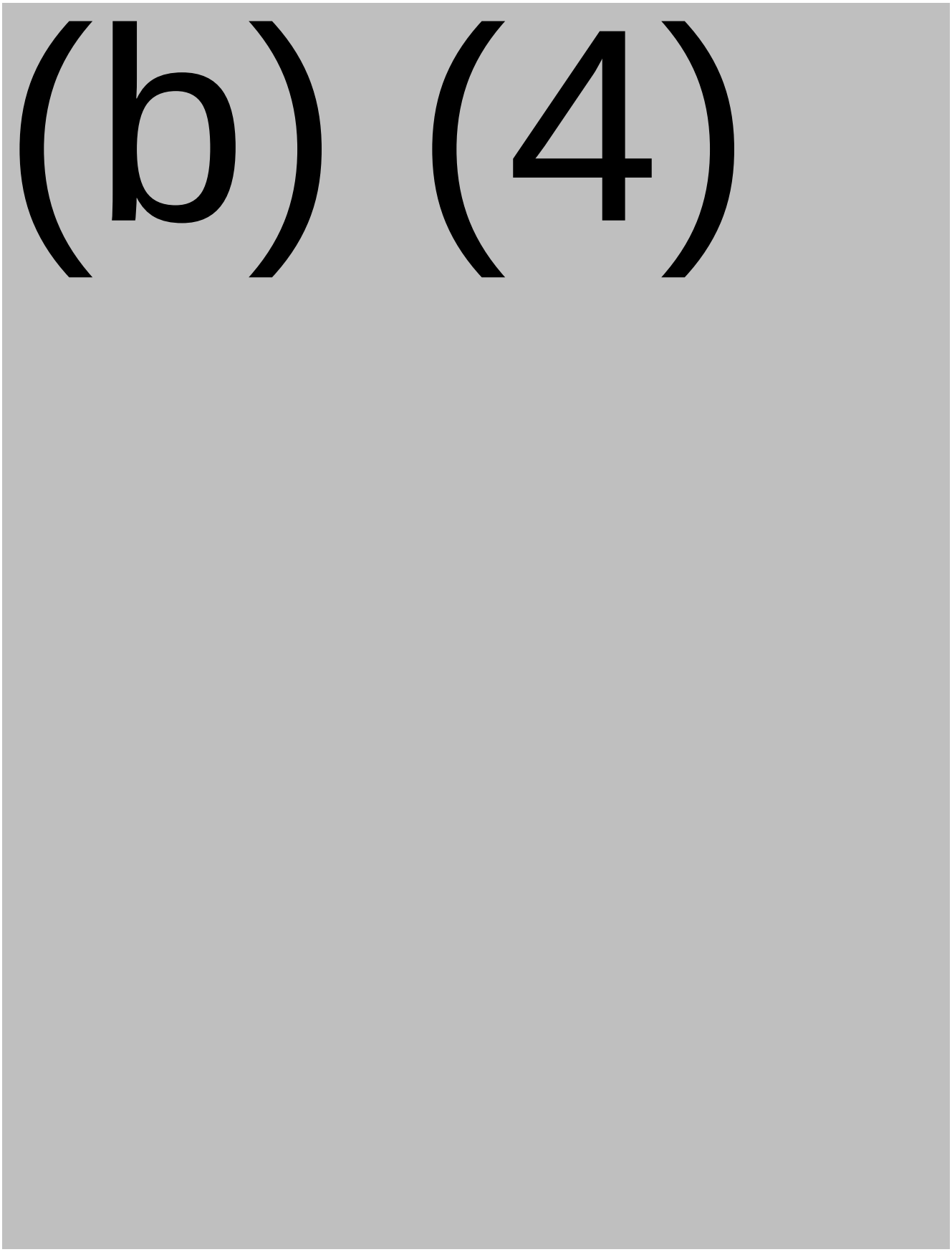

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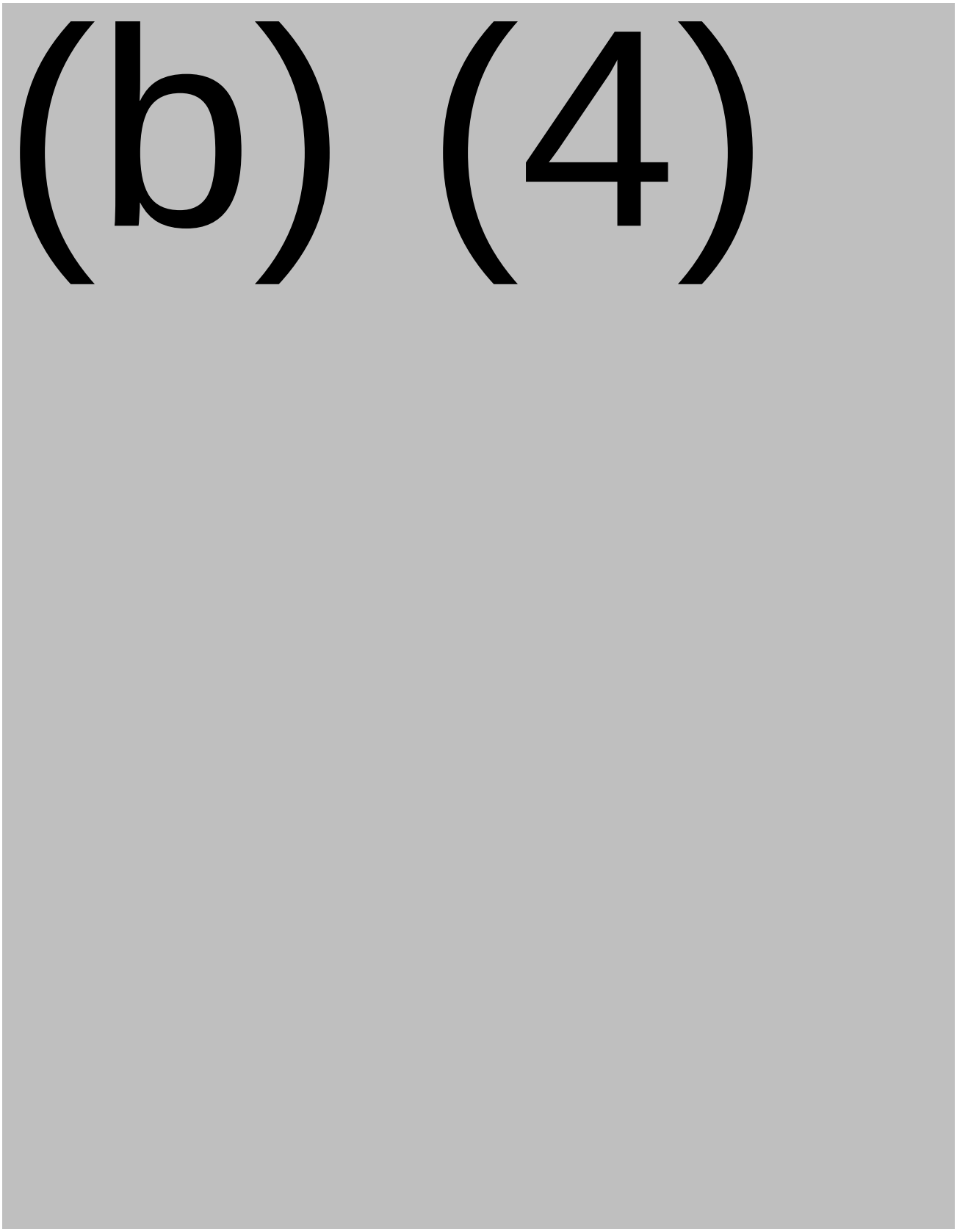

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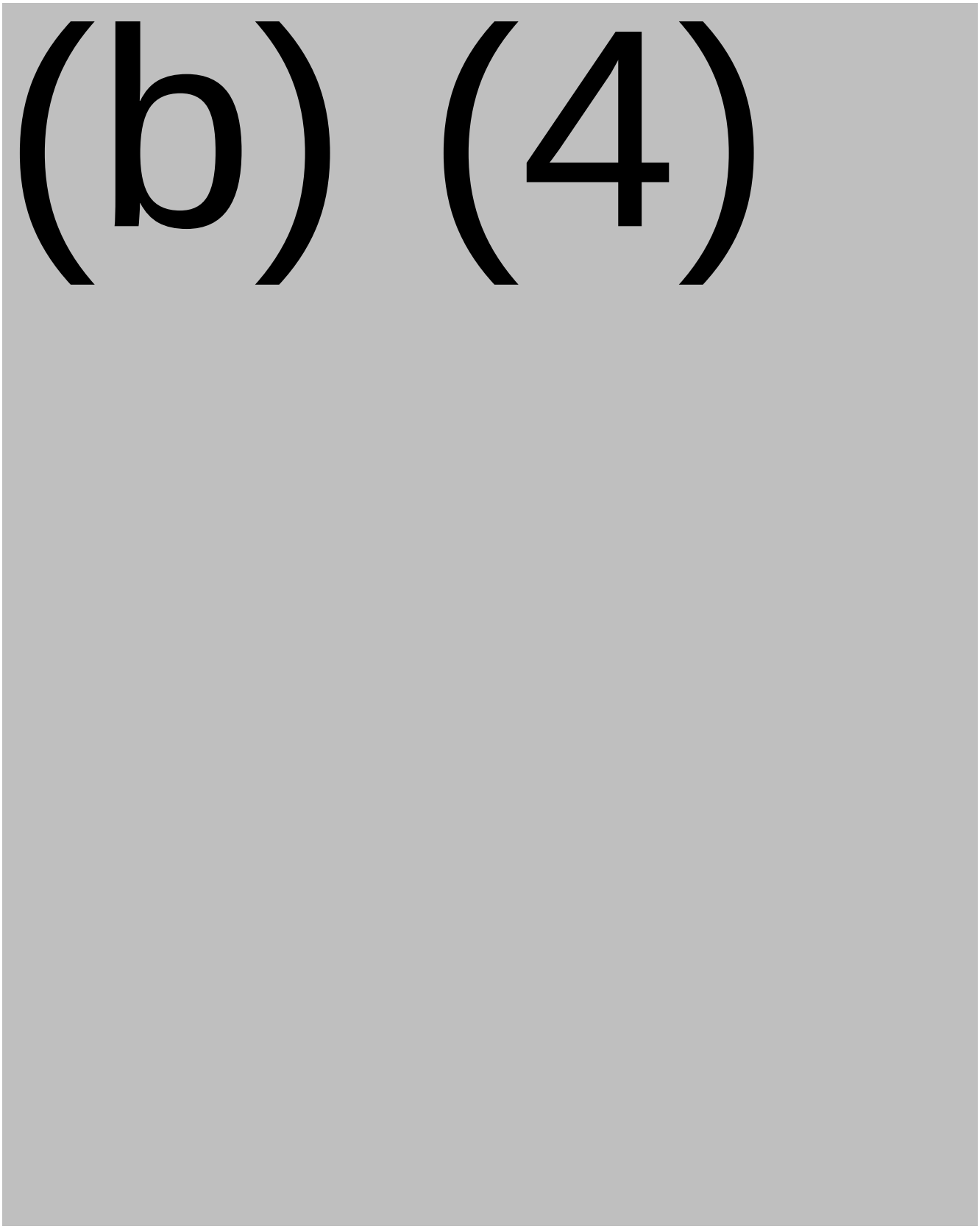

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(b) (4)

Table 7. (b) (4)
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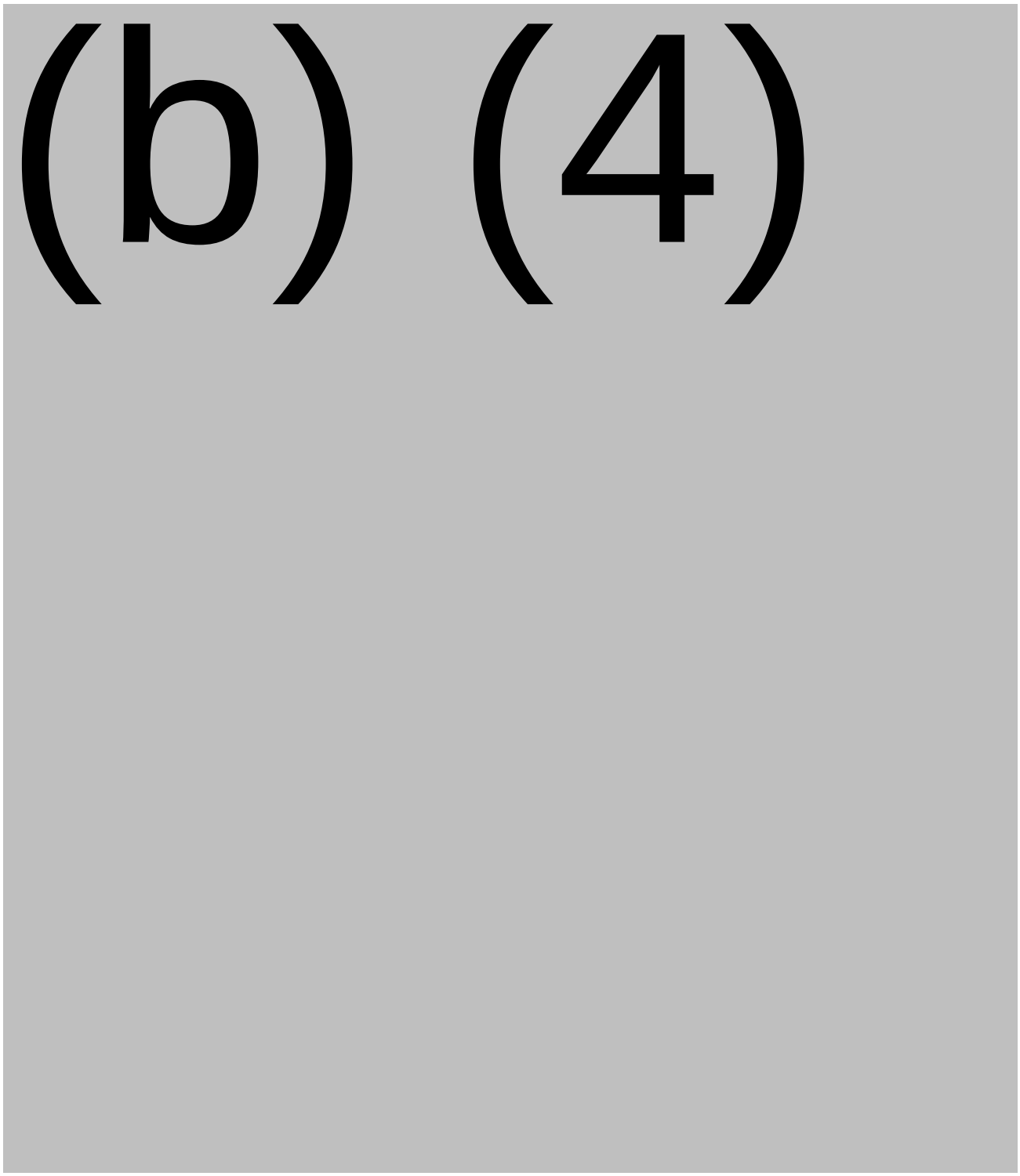

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Table 8. (b) (4)
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G. Analysis

(b) (4)

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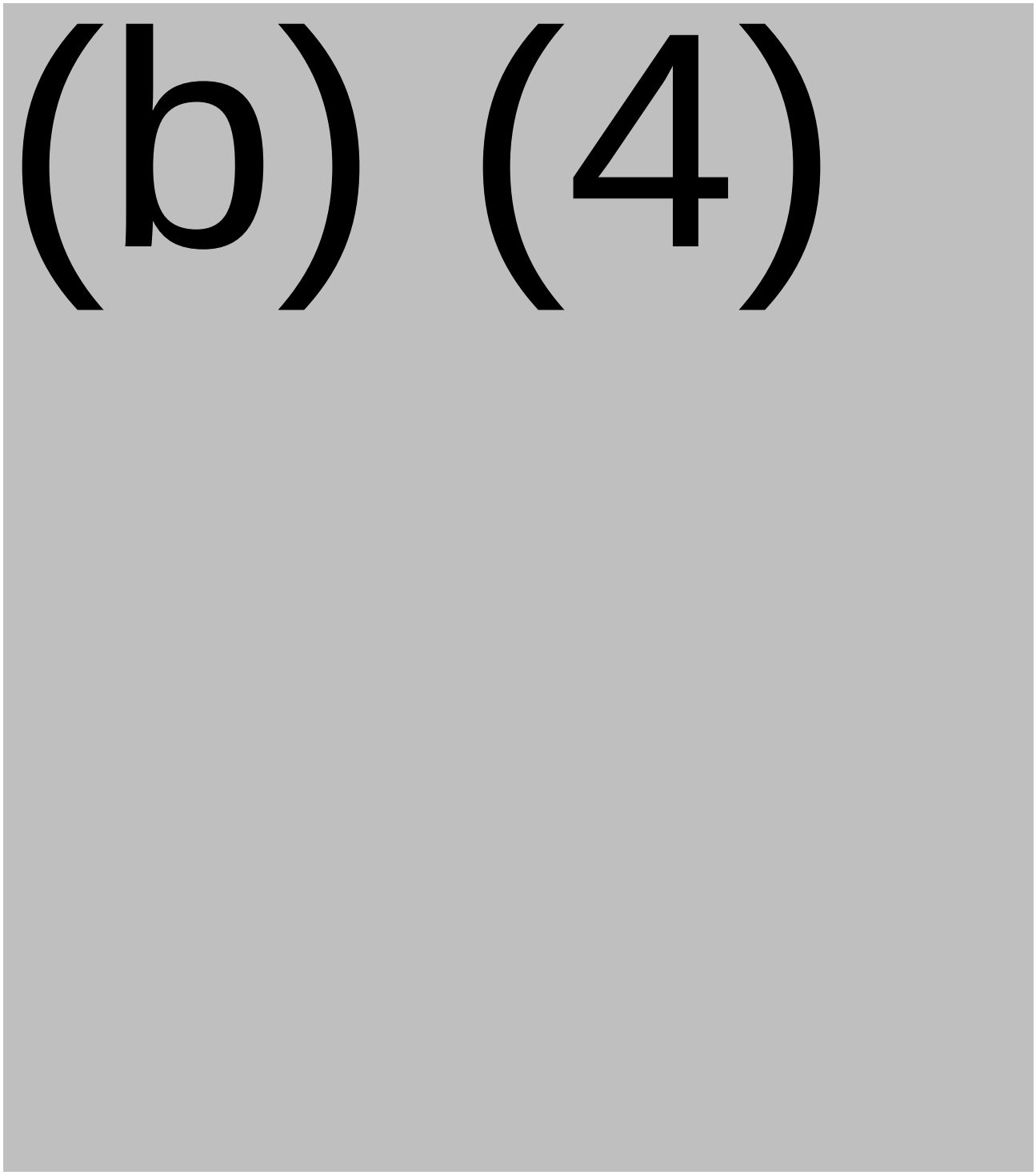

H. Results and Conclusions

Graphical and tabular presentations of results are below. Please see Table 1 for textual descriptions of results. Please see the Methods and Analysis sections for information pertaining to those topics. Detailed methods and results may be found in Binder **BP 2020-040**.

(b) (4)

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(b) (4)

Table 9. (b) (4)

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Table 10. (b) (4)

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Table 11. (b) (4)

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Table 12. (b) (4)

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Table 13. (b) (4)

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Table 14. (b) (4)

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(b) (4)

Table 15. (b) (4)

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Table 16. (b) (4)

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Table 17. (b) (4)

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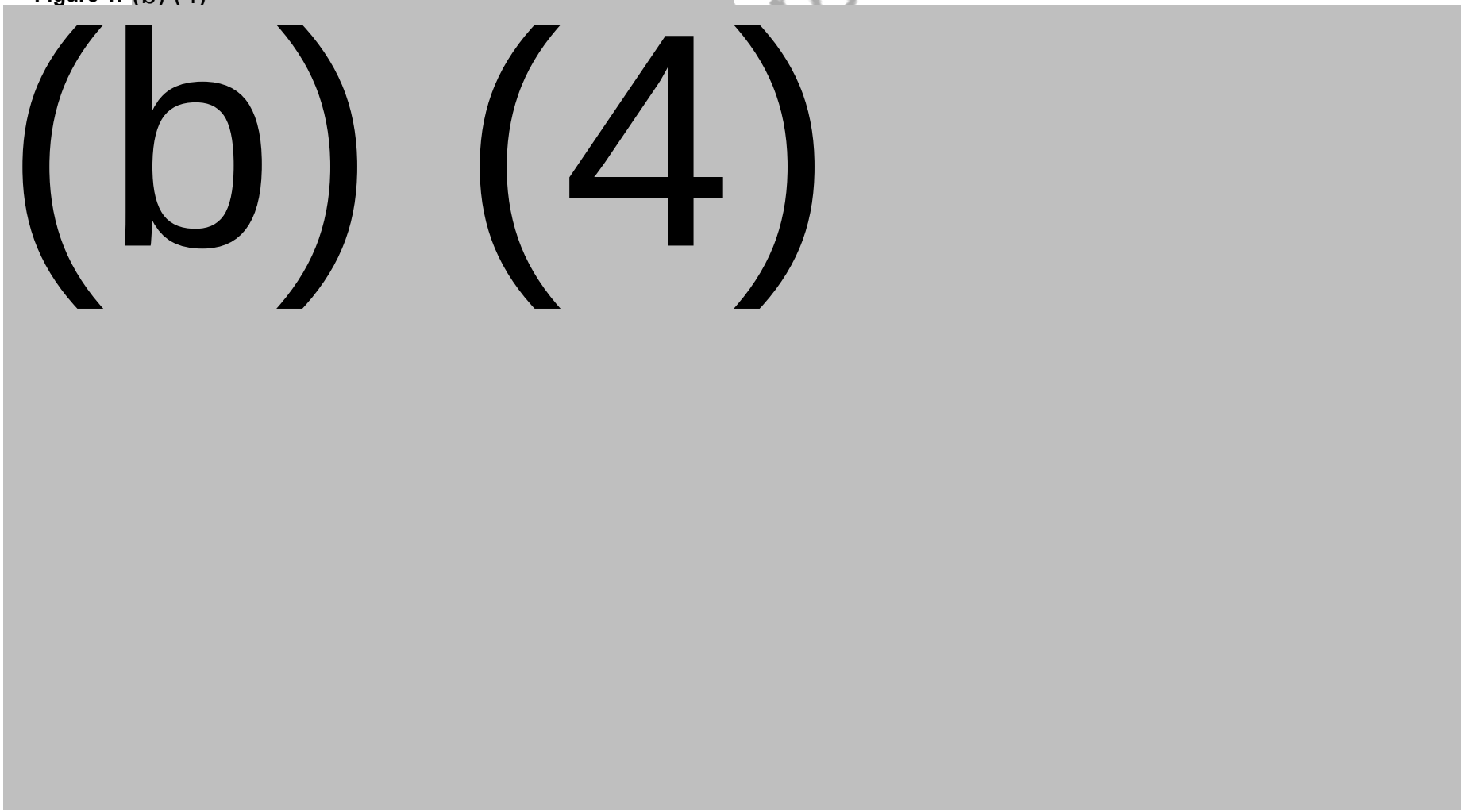
(b) (4)

Figure 1. (b) (4)



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(b) (4)

Figure 2. (b) (4)

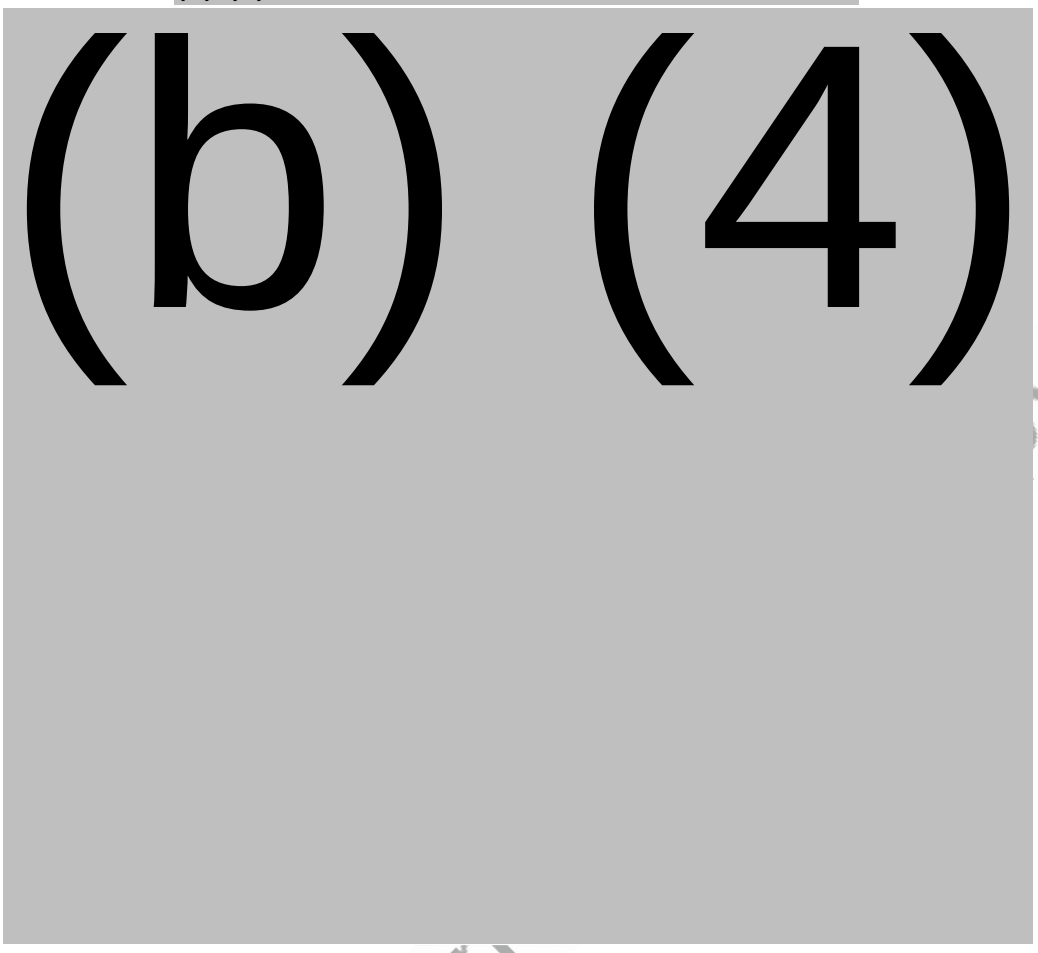

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Figure 3. (b) (4)

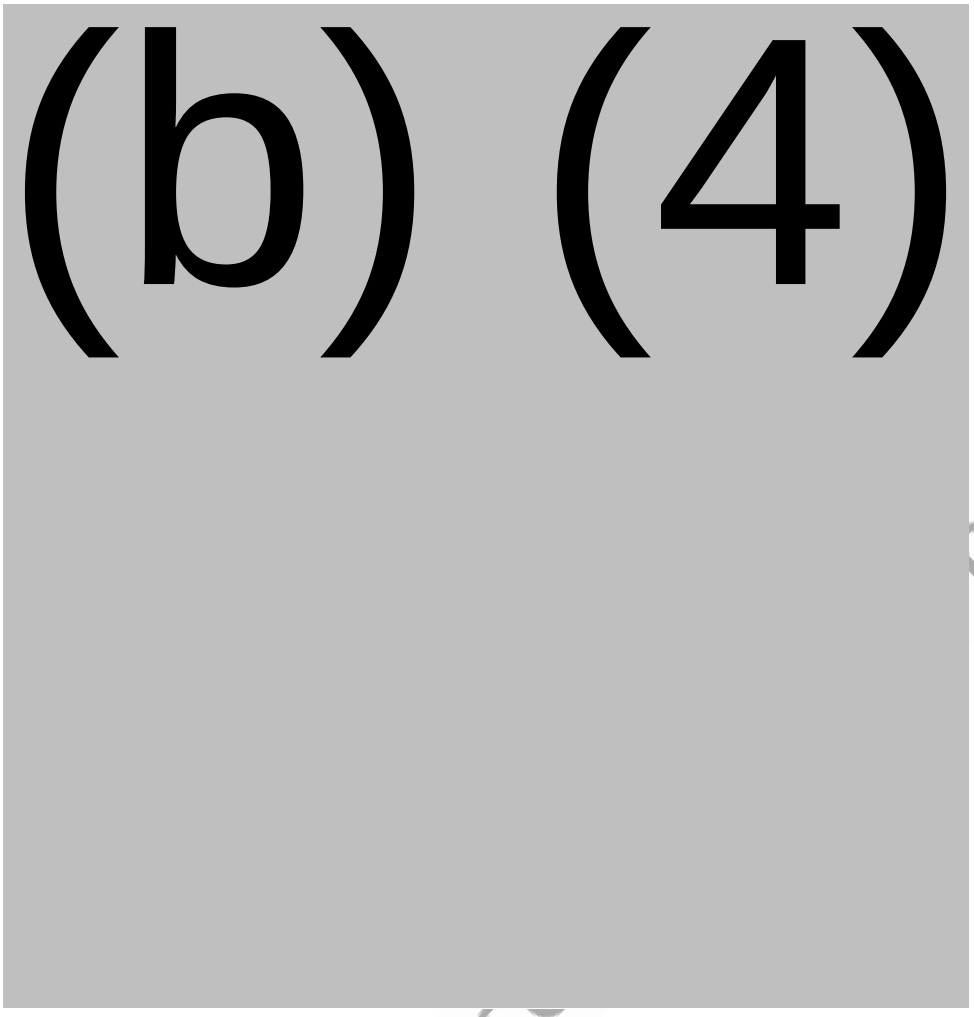

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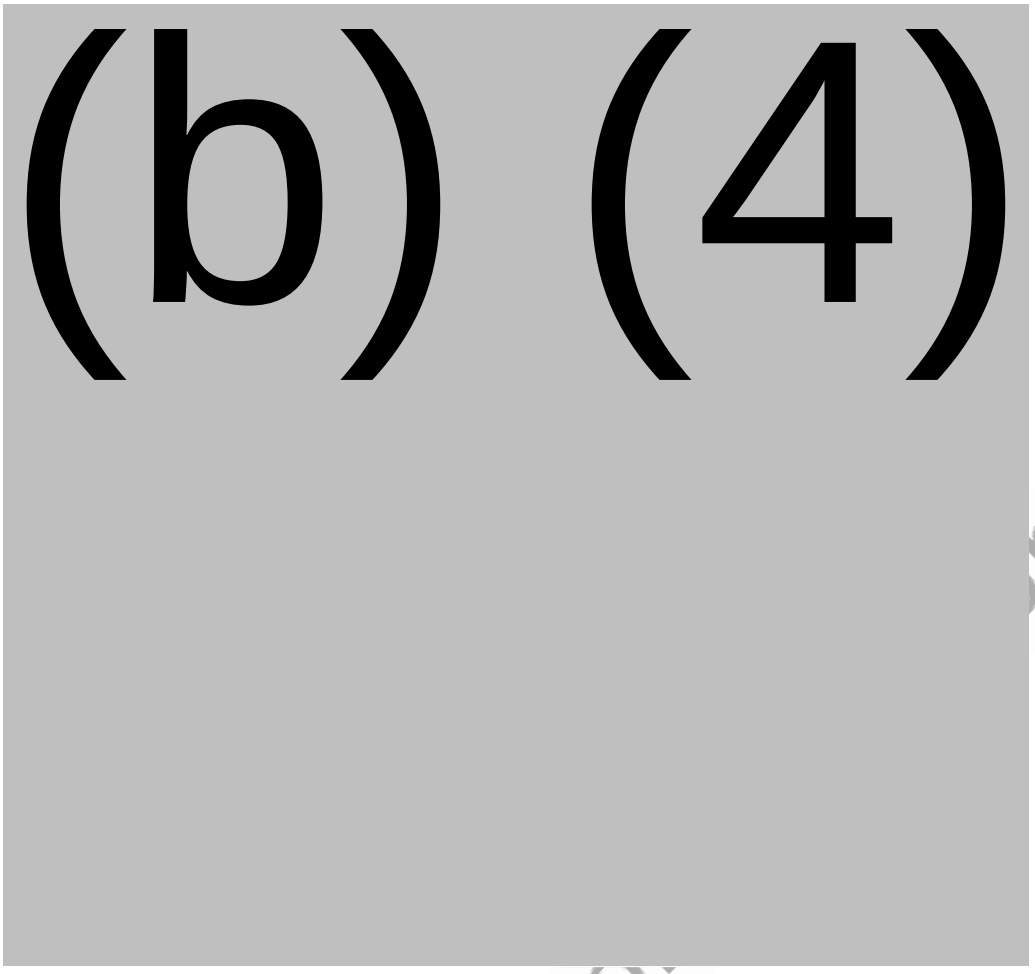
Figure 4. (b) (4)



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Extraction (b) (4)

Figure 5. (b) (4)
(b) (4)

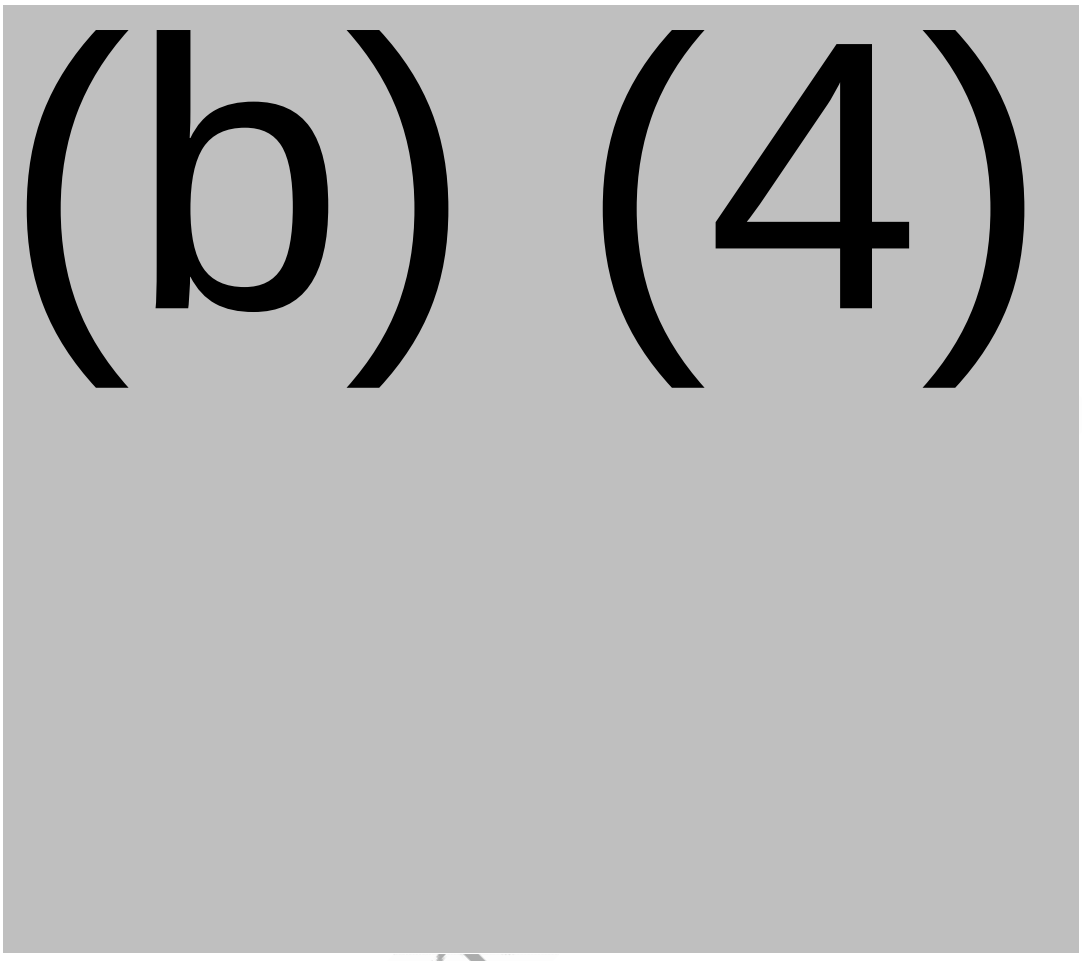

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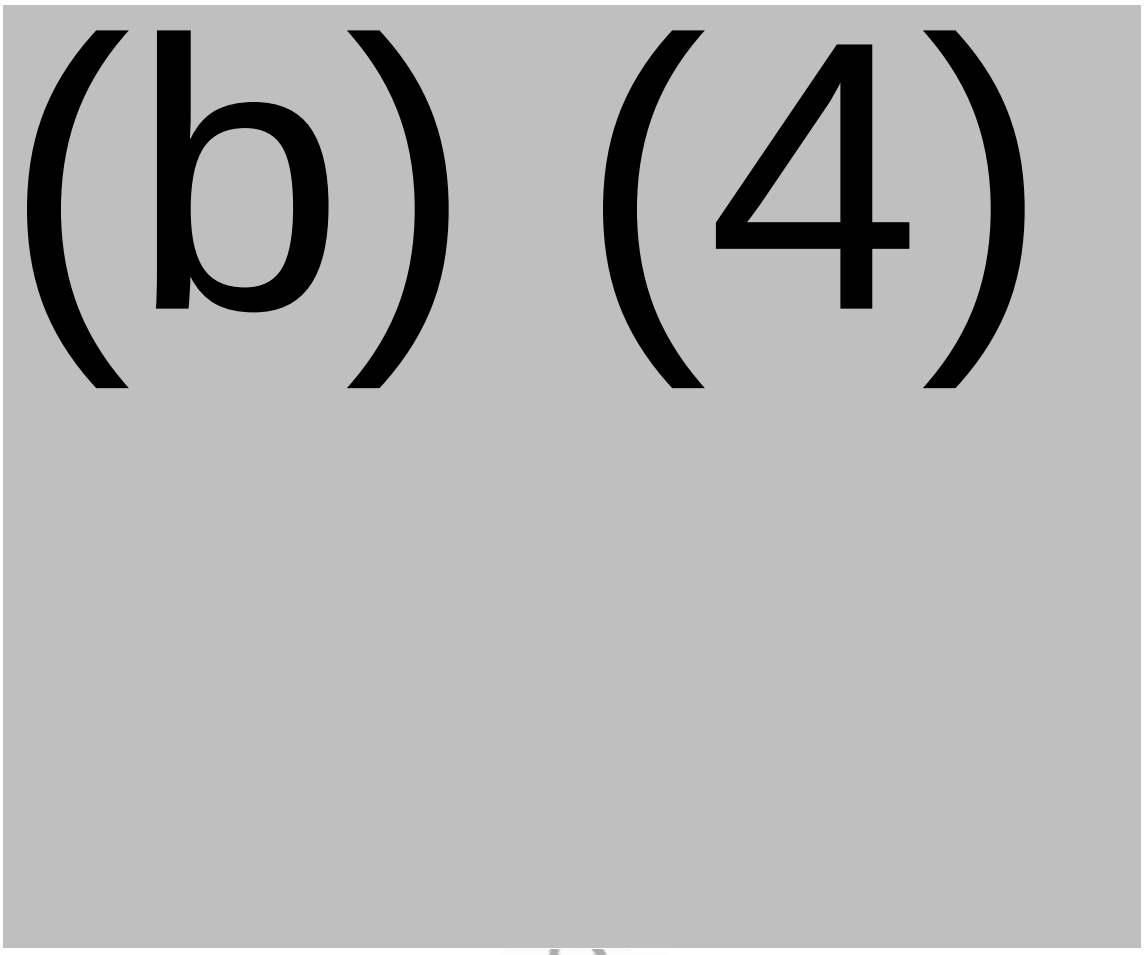
Figure 6. (b) (4)
(b) (4)



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RT-qPCR (b) (4)

Figure 7. (b) (4)
(b) (4)

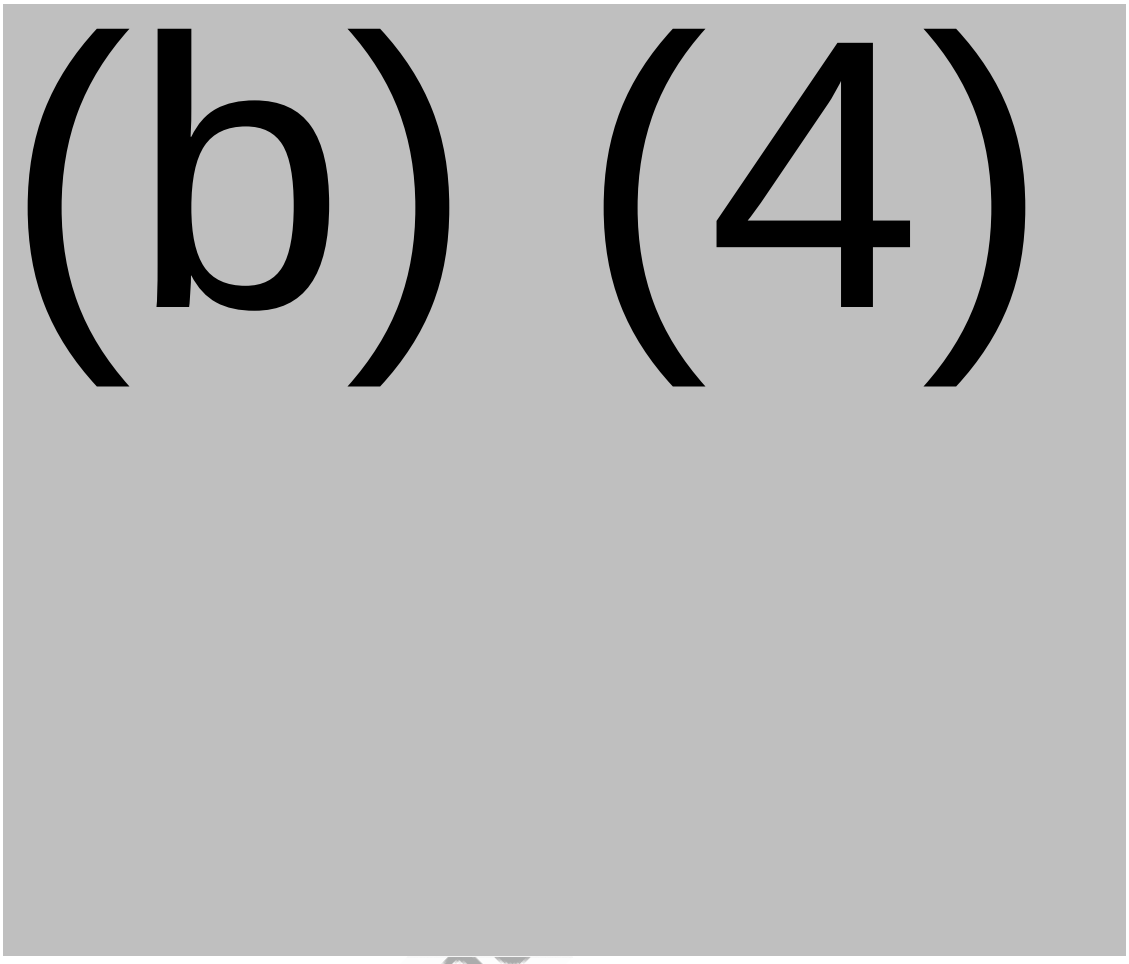

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Figure 8. (b) (4)
(b) (4)

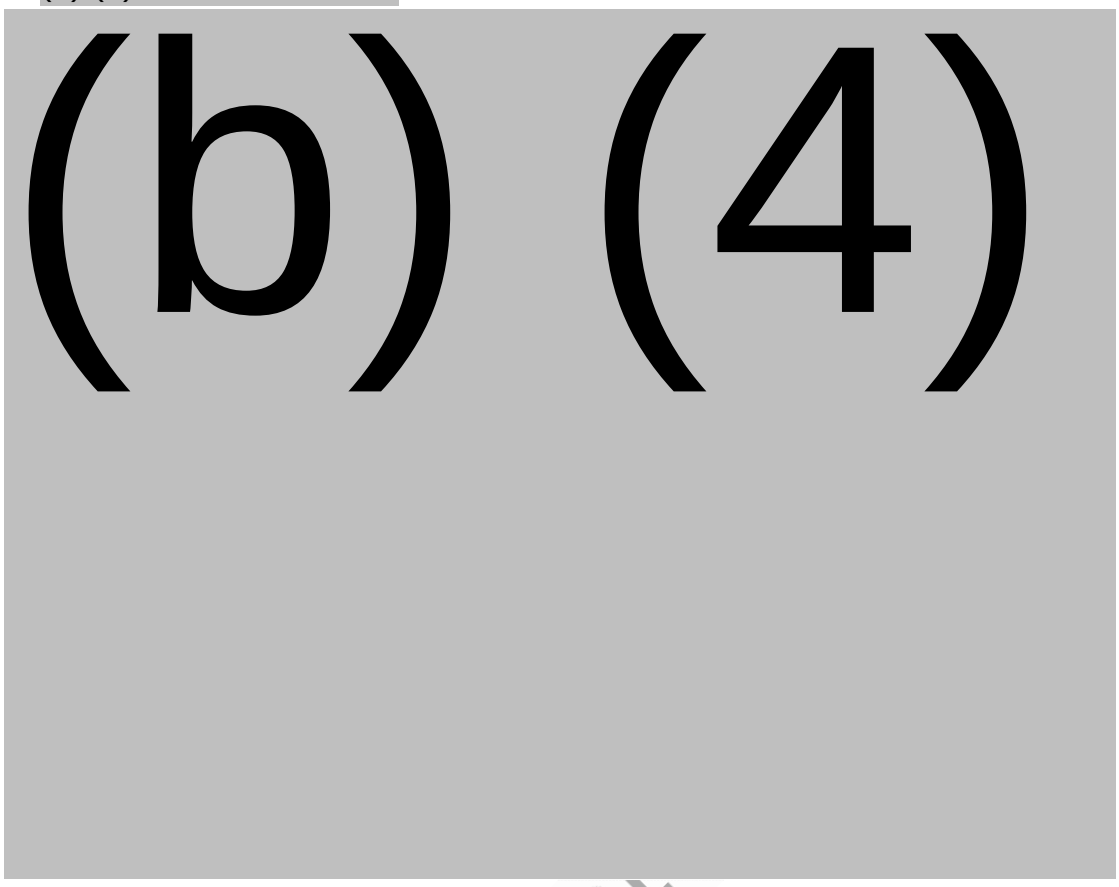

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Table 18. (b) (4)

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(b) (4)

Table 19. (b) (4)

(b) (4)

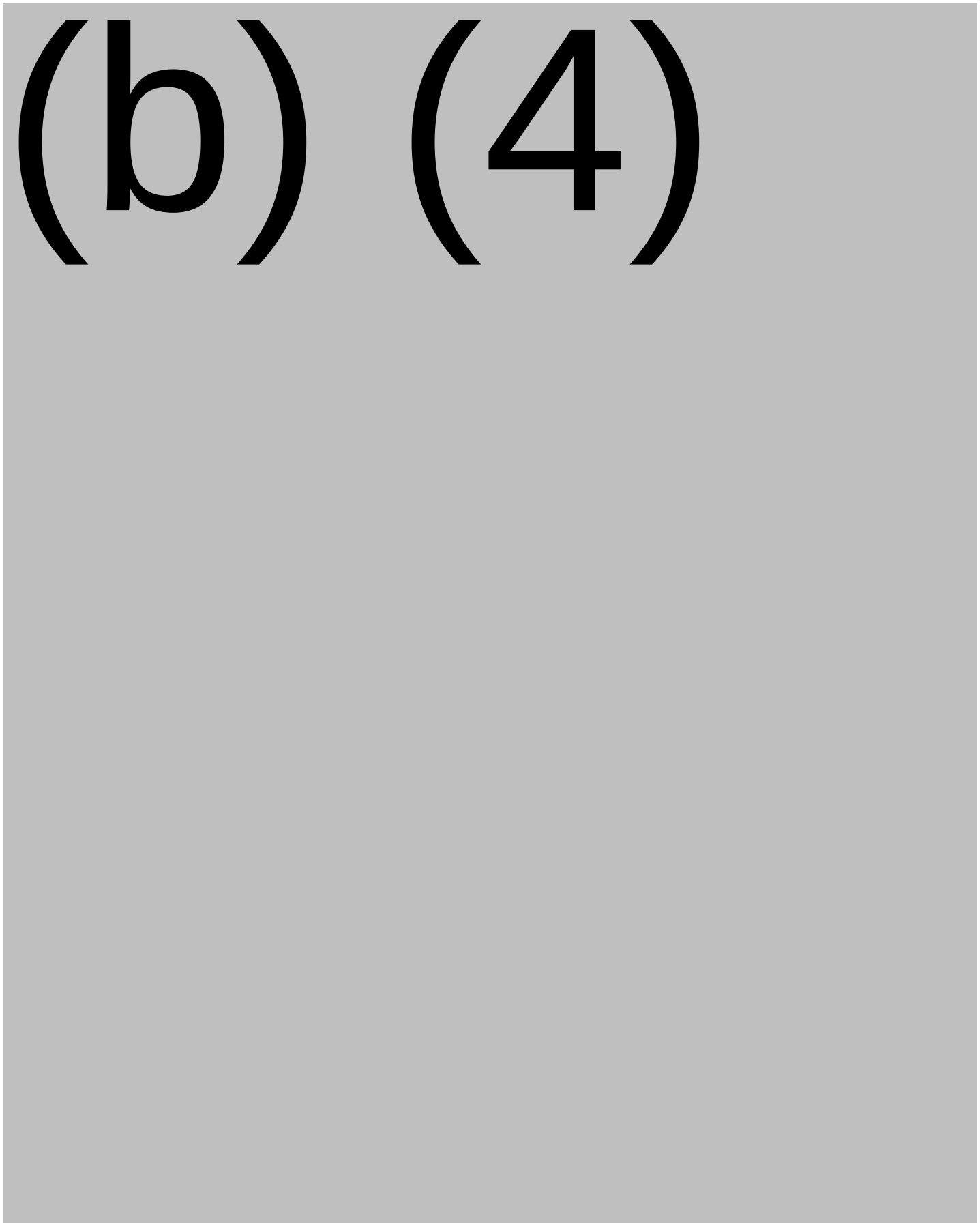

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Table 20. (b) (4)

(b) (4)

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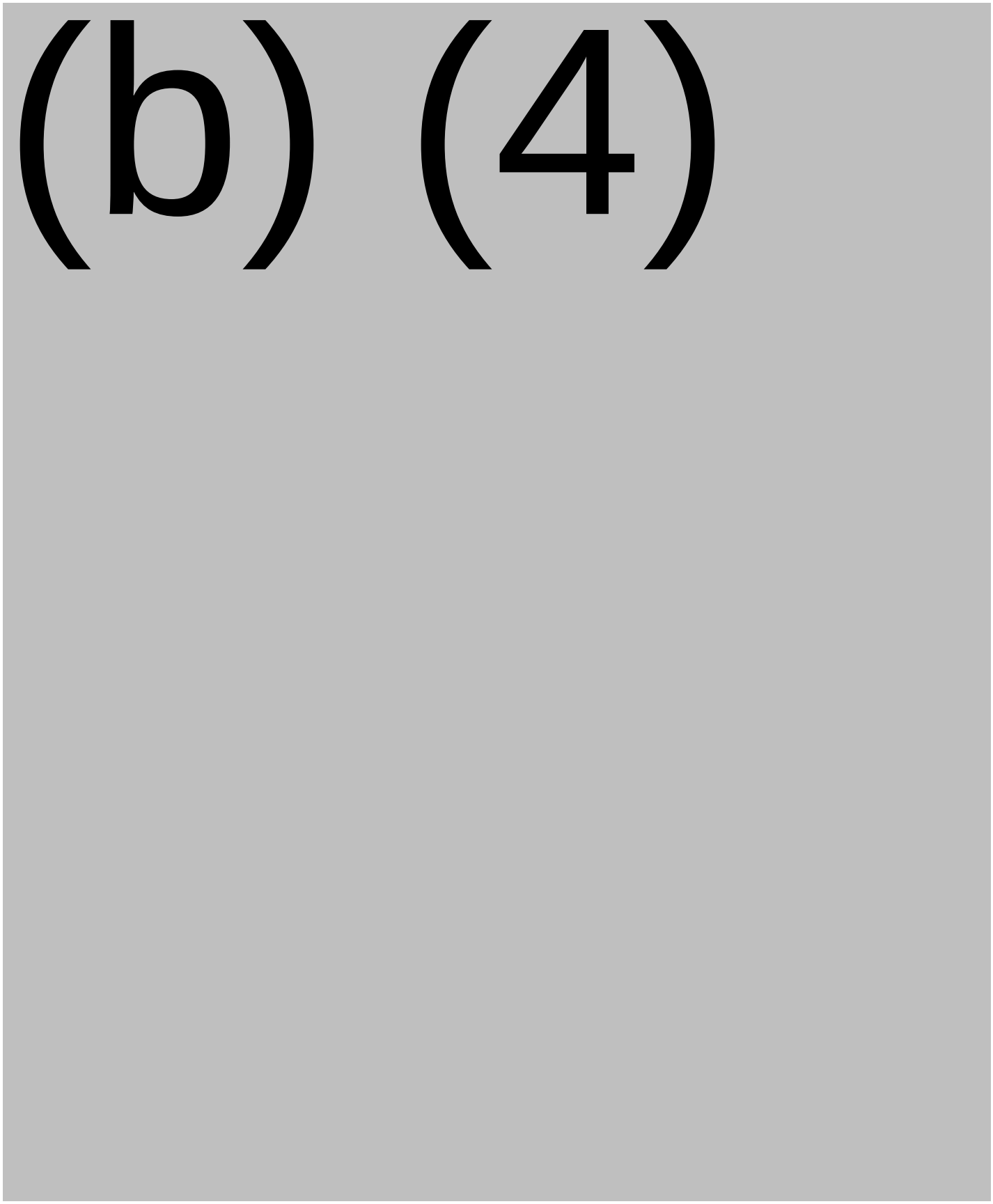

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(b) (4)

Table 23. (b) (4)

(b) (4)

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Table 24. (b) (4)

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Table 25. (b) (4)

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Table 26. (b) (4)

(b) (4)

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Table 27. (b) (4)

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Table 28. (b) (4)

(b) (4)

(b) (4)

Table 29. (b) (4)

(b) (4)

I. Exceptions

1. The validation protocol was erroneously titled a "qualification" protocol. The performance assessment approaches are sufficient to address the validation of a RT-qPCR assay.
2. The validation protocol failed to describe that the (b) (4) for a given (b) (4) not only must meet acceptance criteria described in Table 1 with regards to (b) (4) (b) (4) in the assay. For the SARS-CoV-2 RT-qPCR, this value is (b) (4) The levels showing acceptable (b) (4) (b) (4) (b) (4)
3. During validation, (b) (4) (b) (4) were performed using the incorrect amount of water in the RT-qPCR reactions. (b) (4)

   (b) (4)

   (b) (4) (Tables 23 and 24). Thus the data generated using the reaction with (b) (4) was deemed acceptable and utilized in this validation report. Detailed data can be found in Binder **BP 2020-037**.
4. The validation protocol erroneously indicated the proper division factor for converting copies per mL to copies per reaction was (b) (4). The Methods section now correct reflects the proper division factor of (b) (4)
5. For the original test of the (b) (4) (b) (4)

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(b) (4)

(b) (4) See **Table 11** for more details.

J. References

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(b) (4)

(b) (4)

(b) (4)

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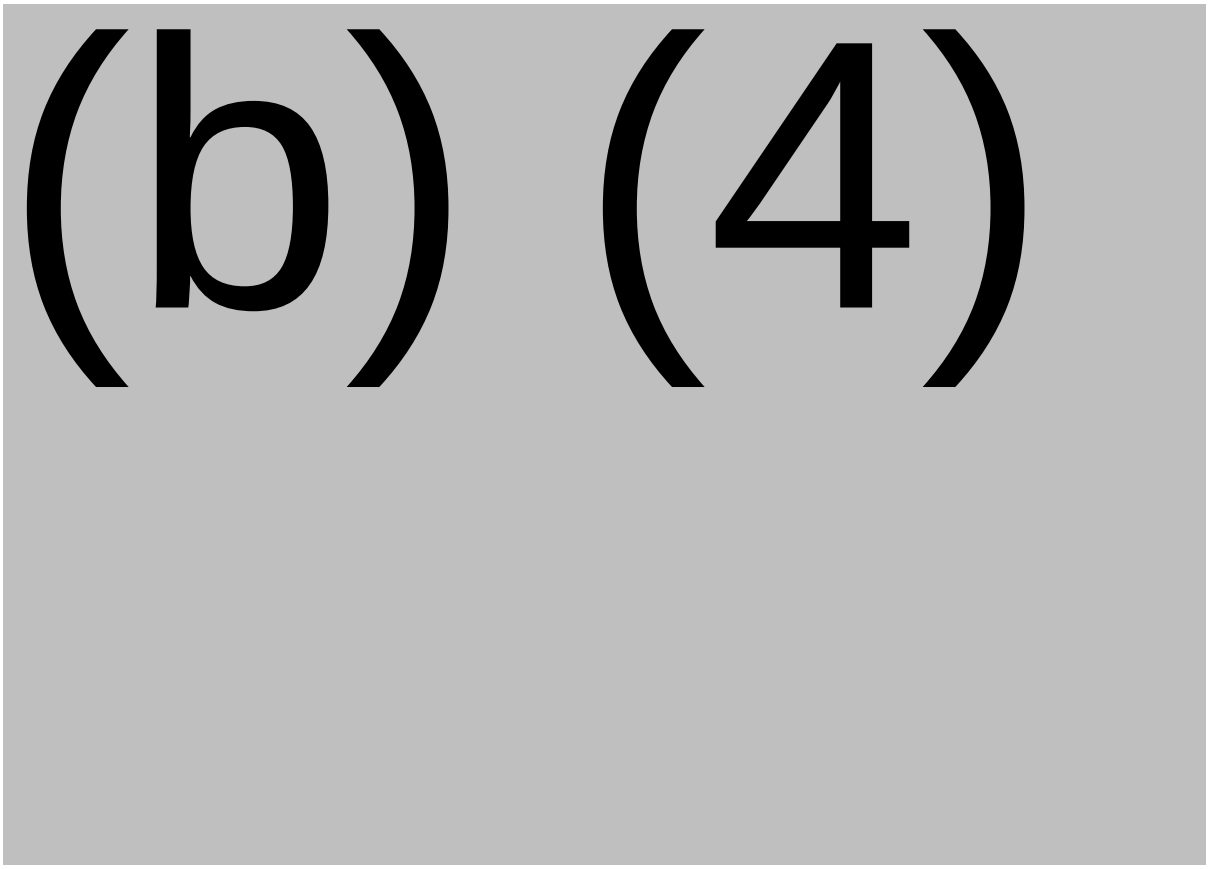
(b) (4)

Submission guidelines for nucleic acid amplification tests for infectious agents, State of New York Department of Health. February, 2011.

K. Appendix

(b) (4) **Assessment**

Figure 9. (b) (4)
(b) (4)



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