

Standardized Growth Protocol for Bacterial Cultures & ODs

FILE NAME: Standardized Growth Protocol for Bacterial Cultures & ODs.doc

**Authored by: Emily Wright
Last Modified on: October 2012
Approved by: Martin Wiedmann**

EFFECTIVE DATE: Date of Approval

APPROVED BY:

Dr. Martin Wiedmann

(date)

AUTHORED BY:

(Name)

(date)

TABLE OF CONTENTS

1.	INTRODUCTION	3
	Purpose	
	Scope	
	Definitions	
	Safety	
2.	MATERIALS	4
3.	PROCEDURE	5
4.	TROUBLESHOOTING	6
5.	REFERENCES	7

SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth standard guidelines for all growth conditions, including RNA extraction and survival experiments.

1.2 Scope

This SOP applies to the Food Safety Lab, including the Laboratory for Food Microbiology and Pathogenesis of Foodborne Diseases.

1.3 Definitions

1.4 Safety

Appropriate protective measures need to be taken when working with bacterial pathogens. All waste from these experiments needs to be treated as BSL-2 waste.

SECTION 2 MATERIALS

- **BHI Media (agar and broth)**

SECTION 3 PROCEDURES

3.1. Standard Protocol for growth conditions

1. Take a single colony from a fresh overnight BHI agar plate and inoculate into 5ml fresh BHI broth. Incubate overnight at desired temperature* with gentle agitation.
2. Add 50mL of the overnight culture to 5ml fresh BHI broth (i.e. a 1/100 dilution) and incubate at desired temperature* with gentle agitation until OD=0.4.
3. Remove a 500 mL aliquot and add to 50 ml of fresh pre-warmed (desired temperature) BHI broth (i.e. a 1/100 dilution) (in a side-arm flask) and incubate at desired temperature* until desired growth phase has been reached:

The following OD readings are now standard for both the Boor and Wiedmann laboratories:

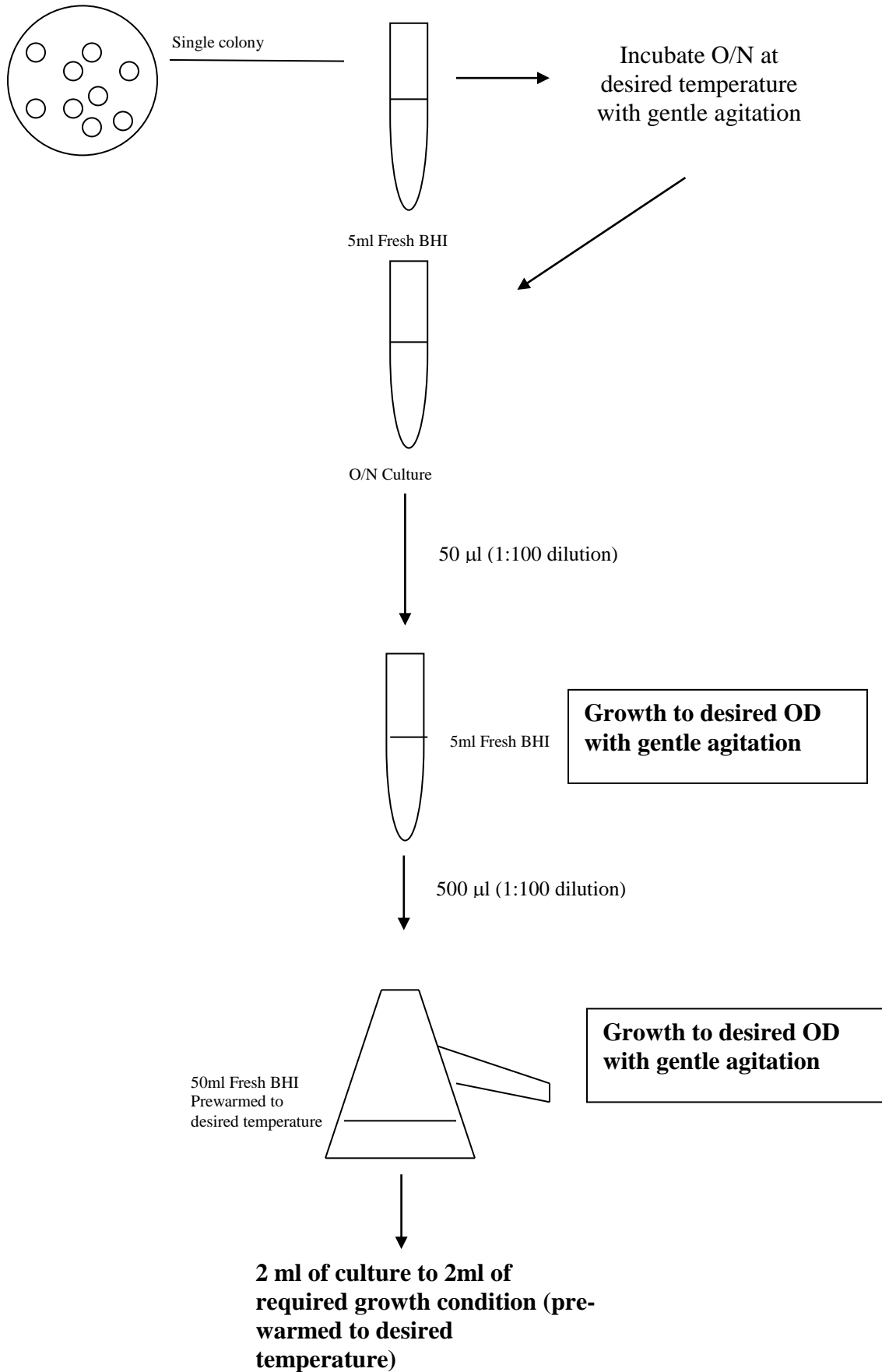
Desired growth phase

	O.D reading
• Early Log Phase	0.4
• Mid Log Phase	1.0
• Late Log/Early Stationary Phase	2.0

Note: When your cultures reach O.D \square 0.5, it is imperative that you continually check your OD readings regularly (every 5-10 minutes should suffice). At this stage the cells are dividing rapidly and the OD increases at a very fast rate.

*Note: The desired temperature can vary from 20C up to 42C. Irrespective of the growth temperature used, follow this protocol but ensure that this temperature is used THROUGHOUT the entire procedure

Schematic of growth conditions



SECTION 4

TROUBLESHOOTING

SECTION 5

REFERENCES

In this section the author should present any publications that were used to construct the protocol or any that might be helpful to the reader.

Example:

Martin B. M. (1994). Tissue Culture Techniques: An Introduction. Boston: Birkhauser.

Note: The sections presented in this template are the minimum requirements for a protocol in CUFSL. Additional sections for organization, procedure timetables, etc... can be included at the author's discretion.