1.1 Protocols of bacterial culture methods for sterility testing of samples from the gnotobiotic unit

1.1.1 Food and bedding samples

Materials

A. Samples from the gnotobiotic unit. These should arrive in 250ml Duran flasks. They must be set up for culture on the day of arrival.
B. Sterilised CM0173 thioglycollate medium CM0173 (450ml in 500ml Duran bottles): you should have one of these bottles for each pair of (sample and control) bottles containing food or bedding from the gnotobiotic unit.
C. Four blood agar plates for each sample will be required after the initial growth period of 7-9 days. Two of these plates (for anaerobic culture) should be available from the anaerobic incubator.

Method

Initial culture

A. Log in the samples in the Excel sheet (GnotformABC.xls where ABC is the three-digit isolator code[1]—see **Gnoto1010** for the codes). Open the Excel file to confirm that the samples with **identical numbers** and the correct sampling date appear in the Connexion Excel file for the appropriate isolator. Note on GnotoformABC.xls and on the lab board the date that the subculture needs to be performed and the date when the final plates should be read. For this you need to consult the calender to avoid unnecessary weekend work. Please write your name on the board for both the subculture and the final reading, and mark up the plates (as in step I) for the subculture and bind them with autoclave tape, so that you are sure that you will have enough on the day (tick the board when you have done this).
B. Print out the current GnotoformABC.xls and place the hard copy showing your samples in the GnotoformABC.xls file.
C. Spray the outside of the samples with 70% alcohol and wipe off the excess carefully. Place the samples in the sterile lamina-flow hood facing inwards to the clean area of the lab.
D. Take one 500ml bottle of CM1073 for each pair of samples and spray with 70% alcohol as before; place in the laminar flow hood as before.
E. Put on gloves and spray your gloved hands with 70% alcohol. Open the tops and add 200ml of CM0173 to each 250 ml bottle. Split one 500ml bottle between a control bottle and a bottle containing a sample.
F. Write, “Culture Started 12 Mar 2005“ on the label of the bottle. **Please do not use any other format for the date such as 12.5.05. or 5.12.05. as this will cause confusion and mistakes.** Place the sample bottles containing the CM0173 into the 37°C incubator.

Sub-culture

G. Prepare 4 blood agar plates for each bottle that is to be subcultured. The anaerobic plates should be taken from at least 24 hours of prior anaerobic conditions. The aerobic plates can be taken from the fridge, but should be placed in the laminar flow hood at least 1 hour beforehand to allow them to warm to room temperature and outer condensation to disperse.

[1] For example, Gnotoform101.xls refers to isolator 101, Gnotoform102.xls refers to isolator 102, and so on.

H. Remove the culture bottles from the 37°C incubator, and spray with 70% alcohol. Put them in the clean laminar-flow hood.
I. Mark the reverse side of two plates with a) the serial number of the bottle to be subcultured and the word 'Anaerobic' b) Culture 12 Mar 2005 c) Subculture 21 Mar 2005 (A or B) and two other plates with the same serial number and the word 'Aerobic' with the dates in the same format.
J. Invert the closed initial culture bottles twice and then loosen the cap. Flame a bacterial loop sequentially and inoculate four plates from each bottle. You will need a demonstration from Andrew on how to do this for the first time.
K. Put aerobic plates in the 37°C incubator and anaerobic plates in the anaerobic incubator.
L. Open GnotoformABC.xls and record the subculture and print out the current sheet and file it. Mark off the lab board that you have done the subculture.

Reading

M. Remove the aerobic and anaerobic plates from the incubators and read the cultures at 48 and 72 hours. Record in GnotoformABC.xls for the appropriate isolator. Tick off the board that you have done the job.
N. Provided that the plates are negative at 72 hours, the original culture bottle can be disposed of. **This is extremely important for good lab management and is the responsibility of the person reading the plates. Check the serial numbers on the plates with the serial numbers on the bottles carefully before proceeding.** Consult Andrew in the event of positive subcultures for a decision on speciation methods.

1.1.2 Swab samples

Materials

A. Samples from the gnotobiotic unit. These should arrive in 100ml Duran flasks. They must be set up for culture on the day of arrival.
B. Prepare the clean laminar flow hood with 70% alcohol spray on the work surfaces
C. Switch on the steriliser until it shows at least 180°C. Place four pairs of forceps in the steriliser.
D. Rack to hold the forceps as they cool on the work surface of the laminar flow hood.
E. Sterilised CM0173 thioglycollate medium CM0173 (12ml in 15ml test tubes): you should have one of these tubes for each sample bottle containing swabs from the gnotobiotic unit.
F. Sterilised Brain-Heart infusion medium CM0225 (12ml in 15ml test tubes): you should have one of these tubes for each sample bottle containing swabs from the gnotobiotic unit.
G. Two blood agar plates for each sample will be required if there is turbidity after incubation. One of these plates (for anaerobic culture) should be available from the anaerobic incubator.

Method

Culture

A. Log in the samples in the Excel sheet (GnotoformABC.xls). Open the Excel file to confirm that the samples with **identical numbers** and the correct sampling date appear in the Connexion Excel file for the appropriate isolator. Note on GnotoformABC.xls and on the lab board the date that the tubes should be read. Please write your name on the board for the final reading, and check that there are plenty of spare blood agar plates (as in step I) for the subculture and write your potential requirement on the board next to the fridge.

B. Spray tubes with 70% alcohol and wipe. Put the tubes in the clean laminar flow hood. For each swab sample bottle mark one tube with the serial number on the bottle and Write ‚Culture started on 2 Mar 2005' on the label of the bottle. **Please do not use any other format for the date such as 2.5.05. or 5.2.05. as this will cause confusion and mistakes.**
C. Remove the forceps from the steriliser and place them on the rack for at least 1 minute to allow them to cool.
D. There will be six samples taken on each isolator connexion. These have the serial number format 102052005, 202052005, 302052005, 402052005, 502052005, 602052005, where the sample number is followed by the date. The first and second samples are controls and have been autoclaved, but not opened in the isolator. The swabs should be tranferred as follows:

Sample	Culture medium
First	CM0173 Thioglycollate
Second	CM0225 Brain Heart Infusion Broth
Third	CM0173 Thioglycollate
Fourth	CM0225 Brain Heart Infusion Broth
Fifth	CM0173 Thioglycollate
Sixth	CM0225 Brain Heart Infusion Broth

E. Open the appropriate sample bottle and culture tube and take one of the sterilised forceps that have cooled to transfer the material from the bottle into the tube. Close the bottle and seal the tube. Continue until all the samples have been set up for culture.
F. Place all the tubes in a rack in the 37°C incubator.
G. Rinse out all the sample bottles and remove the autoclave tape. Place them in the glasswasher.
H. Write on the board that you have done the job and note the date on which the samples should be read (after 7-9 days). Consult the calender so that this is set to avoid unnecessary weekend work. **It is your responsibility to read the samples or to arrange an alternate if you are to be away. It is also important to watch the samples in the interim and proceed with a contaminated (turbid) sample immediately. If there is any doubt, record a contaminated reading in GnotoformABC.xls for the appropriate isolator, because this will automatically alert those managing the isolator that it is potentially contaminated. If the samples later turn out to be negative it is easy to alter this alert.**

Reading

I. Remove the tubes from the incubator on the designated day and read whether or not there is turbidity in the cultures. Record in GnotoformABC.xls for the appropriate isolator. Tick off the board that you have done the job.
J. Provided that the tubes are negative at 9 days, they can be disposed of. **This is extremely important for good lab management and is the responsibility of the person reading the samples.**
K. **Samples that are turbid (positive) should be retained.** These should be subcultured aerobically and anaerobically on blood agar plates (see steps G to M of the fodd and bedding sample protocol above for specifics of how to do this). Consult Andrew in the event of positive plates for a decision on speciation methods.

1.1.3 Watersamples

1.1.3.1 Determination of the total bacterial population of water samples

Materials

A. Samples from the gnotobiotic unit. These should arrive in 500ml Duran flasks. They must be set up for culture on the day of arrival.
B. Prepare the clean laminar flow hood with 70% alcohol spray on the work surfaces
C. One CM0129 CASO Agar plate per sample bottle to be tested
D. One CM0579 Pseudomonas selective Agar plate per sample bottle to be tested
E. One new sterile 50ml syringe per sample to be tested.
F. Two new sterile 16 gauge needles per sample to be tested.
G.
H. Sterilised CM0173 thioglycollate medium CM0173 (12ml in 15ml test tubes): you should have one of these tubes for each sample bottle containing swabs from the gnotobiotic unit.
I. Sterilised Brain-Heart infusion medium CM0225 (12ml in 15ml test tubes): you should have one of these tubes for each sample bottle containing swabs from the gnotobiotic unit.
J. Two blood agar plates for each sample will be required if there is turbidity after incubation. One of these plates (for anaerobic culture) should be available from the anaerobic incubator.

Method

Culture

K. Log in the samples in the Excel sheet (GnotoformABC.xls). Open the Excel file to confirm that the samples with **identical numbers** and the correct sampling date appear in the Connexion Excel file for the appropriate isolator. Note on GnotoformABC.xls and on the lab board the date that the tubes should be read. Please write your name on the board for the final reading, and check that there are plenty of spare blood agar plates (as in step I) for the subculture and write your potential requirement on the board next to the fridge.

Medium:	CASO-Agar
Verdünnungsstufen:	100 ml Originalwasser
Technik:	Membranfiltertechnik mit 0.45 mm Filter; der Filter wird auf den Agar aufgelegt.
Bebrütung:	3-5 Tage bei 30°C
Auswertung:	Auszählen der sichtbaren Kolonien.

1.1.3.2 Bestimmung von Pseudomonaceae und P. aeruginosa

Medium:	Pseudomonas-Cetrimid-Selektiv Agar CM0579
Verdünnungsstufen:	100 ml Originalflüssigkeit
Technik:	Membranfilter-Technik mit 0.45 mm Filter; die Filter werden auf den Agar aufgelegt.
Bebrütung:	24 - 48 Std. bei 37°C
Auswertung:	Auszählen der sichtbaren Kolonien und bestätigen, ob Pseudomonaceae oder P. aeruginosa gewachsen sind.