3.17 Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Emerging Infectious Diseases (EIDs)

Purpose

To provide clinical laboratory personnel with information to protect themselves from exposure to Emerging Infectious Diseases (EIDs) while handling and processing specimens from EID patients.

Audience

Clinical laboratory personnel (faculty/residents/staff).

Policy

I. Personnel will adhere to CDC guidelines outlined in this policy when handling and processing specimens labeled as being from EID patients.

CDC Guidelines

A. Clinical laboratories performing routine hematology, urinalysis, and clinical chemistry studies, and microbiology laboratories performing diagnostic tests on serum, blood, or urine specimens should follow standard laboratory practices, including Universal Precautions, when handling potential EID specimens. For additional information, see www.osha.gov/SLTC/bloodbornepathogens/index.html#revised_standard

B. Microbiology and pathology laboratories performing diagnostic tests on stool or respiratory specimens should handle potential EID specimens using standard Biosafety Level (BSL)-2 work practices in a Class II biological safety cabinet (BSC).

Handling and Processing Specimens

II. Biosafety guidelines for handling EID specimens, by specimen type, are provided below.

A. General precautions when working with specimens that contain an EID agent or that may contain an EID agent.

1. Work surfaces should be decontaminated on completion of work with appropriate disinfectants. All disposable waste should be autoclaved.
2. Laboratory workers should wear personal protective equipment (PPE), including disposable gloves and laboratory coats.
3. Any procedure or process that cannot be conducted in a BSC should be performed while wearing gloves, gown, goggles and a fit tested N-95 mask.
4. Acceptable methods of respiratory protection include: a properly fit-tested, NIOSH-approved filter respirator (N-95 or higher level) or a powered air-purifying respirator

(PAPR) equipped with high-efficiency particulate air (HEPA) filters. Accurate fit-testing is a key component of effective respirator use.[1] Personnel who cannot wear fitted respirators because of facial hair or other fit limitations will wear a PAPR.

Appropriate physical containment devices (e.g. centrifuge safety cups; sealed rotors) should also be used. Rotors and cups should be loaded and unloaded in a BSC.

Note: Packaging, shipping, and transport of specimens from possible and known cases of an EID must follow the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations at (www.iata.org/dangerousgoods/index) and US DOT 49 CFR Parts 171-180 (hazmat.dot.gov/rules.htm). Step-by-step instructions on appropriate packaging and labeling are provided at: www.cdc.gov/ncidod/sars/pdf/packingspecimens-sars.pdf

B. All specimens from EID patients will arrive at the laboratories in a plastic bag with an ABP (yellow) sticker on the outside of the bag.

C. Storage of any specimen for future testing must be done with the utmost caution. The specimen should be placed in a disposable rack and the rack placed into a biohazard bag and the bag sealed. A yellow ABP sticker should be placed on the outside of the bag. Place the bag in the appropriate refrigerator or cabinet in such a way as to minimize the risk of the specimen container tipping over.

D. When specimens are taken out of the bag on arrival to the laboratory, the outside of the container should be wiped with a disinfectant.

E. Blood (blood, serum and plasma) and urine specimens

1. Handle these specimens using All Barrier Precautions (APB), which includes use of gloves, gown, N-95 mask, and eye protection.

2. Any procedure with the potential to generate fine-particulate aerosols (e.g., vortexing or sonication of specimens in an open tube) should be performed in a biological safety cabinet (BSC). Use sealed centrifuge rotors or sample cups for centrifugation. Rotors and cups should be loaded and unloaded in a BSC. Perform any procedures outside a BSC in a manner that minimizes the

risk of exposure to an inadvertent sample release.

3. After specimens are processed, decontaminate work surfaces and equipment. Use any EPA-registered hospital disinfectant. Follow manufacturer's recommendations for use-dilution (i.e., concentration), contact time, and care in handling.
4. See Appendices A, B, and C for Hematology, Chemistry and Blood Bank, respectively.

F. Other specimens (e.g., respiratory secretions, stool, or tissue for procedures performed in microbiology or pathology laboratories). See Appendix D – Microbiology.

1. The following activities may be performed in BSL-2 facilities with standard BSL-2 work practices:
   a. Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues
   b. Molecular analysis of extracted nucleic acid preparations
   c. Electron microscopic studies with glutaraldehyde-fixed grids
   d. Routine examination of bacterial and mycotic cultures
   e. Routine staining and microscopic analysis of fixed smears
   f. Final packaging of specimens for transport to diagnostic laboratories for additional testing. Specimens should already be in a sealed, decontaminated primary container.
2. The following activities involving manipulation of untreated specimens should be performed in BSL-2 facilities and in a Class II BSC:
   a. Aliquoting and/or diluting specimens
   b. Inoculating bacterial or mycological culture media
   c. Performing diagnostic tests that do not involve propagation of viral agents in vitro or in vivo
   d. Nucleic acid extraction procedures involving untreated specimens
   e. Preparation and chemical- or heat-fixing of smears for microscopic analysis

G. Transfusion Service

1. Cells and plasma will be separated by a Blood Bank staff member. The designated Blood Bank staff member will wear All Barrier Protection (ABP) gear (N-95 mask, goggles, gown and gloves) and use equipment in the Transfusion Service area to process the sample(s) in a manner that minimizes the risk of exposure from

aerosolization or spills.

2. Samples will be loaded on the Provue to determine ABO and Rh and to perform the antibody screen.
3. If the antibody screen is positive, set up a separate workstation in the back of the lab to minimize contamination of staff and laboratory instruments.
4. Determine the specificity and perform antigen typing and extended crossmatch as indicated.
5. Save the specimens for storage in a separate, clearly marked biohazard bag.
6. After specimens are processed, decontaminate all equipment and work surfaces with disinfectant. Run bleach solution through the cell washers to disinfect the tubing.

H. Autopsy service and surgical pathology

Objective: Safely handle human tissue to prevent transmission of an EID. At the discretion of the attending pathologist, an open autopsy or a closed organ biopsy procedure may be performed. The full list of precautions below will be used for whichever procedure is chosen.

1. Autopsy
   a. In general, safety procedures for human remains infected with an EID should be consistent with those used for any autopsy procedure. However, additional respiratory protection is needed during an autopsy procedure that generates aerosols (e.g., use of oscillating saws).
   b. Personal protective equipment (PPE)
      1) Wear standard autopsy PPE, including a scrub suit worn under an impervious gown or apron, eye protection (i.e., goggles, face shield), double surgical gloves with an interposed layer of cut-proof synthetic mesh gloves, a fit tested N-95 mask, and disposable shoe covers.
      2) Autopsy personnel who cannot wear a disposable particulate respirator because of facial hair or other fit limitations will wear a PAPR.
      3) If generation of a high concentration of an aerosol is unavoidable, the operator(s) must wear a PAPR. See Policy 3.8 Protection During the Conduct of High-Risk Respiratory Procedures in Patients with an Emerging Infectious Disease (EID).
      4) Remove PPE before leaving the autopsy suite and dispose in accordance with facility policies and procedures. See Appendix F.

c. Engineering controls
   1) Whenever possible, perform autopsies on human remains infected with an EID in autopsy settings that have an adequate air-handling system. This includes a minimum of 6 (old construction) to 12 (new construction) ACH, negative pressure relative to adjacent areas as per recommendations for AIIRs, and direct exhaust of air to the outside or passed through a HEPA filter if air is recirculated. Exhaust systems around the autopsy table should direct air (and aerosols) away from healthcare workers performing the procedure (e.g., exhaust downward).
   2) Use containment devices whenever possible. Use Biosafety cabinets for the handling and examination of smaller specimens. When available, use vacuum shrouds for oscillating saws to contain aerosols and reduce the volume released into the ambient air environment. See Appendix E.

d. Prevention of percutaneous injuries
   Follow standard safety procedures for preventing percutaneous injuries during autopsy.

2. Surgical Pathology
   a. All specimens will be received in formalin and allowed to sit 1 hour/mm of thickness before they will be processed.
   b. If a fresh specimen is sent, it will be taken to the AFB laboratory in Microbiology and a section cut under the hood and placed in alcohol.
   c. The rest will be placed in formalin.
   d. Specimens may then be transported to the Surgical Pathology Laboratory.
   e. See Appendix F – Surgical Pathology

I. Medical Surveillance Guidelines (CDC)
   1. Laboratory workers should receive training on the appropriate Biosafety level for the type of work being performed.
   2. Before working with either live EID microorganisms or clinical specimens known to contain an EID microorganism, laboratory workers should have a baseline serum sample obtained and stored for future reference.
   3. Laboratory workers who process specimens from patients with an EID will take their temperatures twice per day. Each laboratory worker who processes a specimen(s)

from EID patients will daily record their temperature and check "yes" or "no" to a list of symptoms on a monitoring form (see Appendix G). Employees who develop a fever (100.4° F or 38° C) and have two or more symptoms listed on the monitoring form, if at work will immediately notify their supervisor, don a surgical mask, perform hand hygiene and leave their work site or if at home, will remain at home and call their supervisor.

4. Laboratory workers who are believed to have had a laboratory exposure to an EID should notify their supervisor who should notify Healthcare Epidemiology immediately 24/7 at 23192 during working hours and on pager 643-3133 after hours.

J. Management of a Break in Laboratory Procedure
In the event of an identifiable break in laboratory procedure (e.g., tear in a glove; spill of live virus), the laboratory worker should immediately implement applicable laboratory procedures for emergency exposure management and/or environmental decontamination and notify the supervisor for further instructions. The worker and the supervisor, in consultation with the Employee Health Service (EHS) and the Department of Healthcare Epidemiology, should evaluate the break in procedure. Call the Department of Healthcare Epidemiology at 23192 or on pager (409) 643-3133 after hours 24/7.

K. Management of Exposed Laboratory Workers

1. **If a laboratory worker has been exposed to avian or swine influenza, as determined by the Department of Healthcare Epidemiology, they will be directed to report to the Employee Health Service during regular hours or to the Emergency Department at other times 24/7. They will receive prophylaxis with oseltamavir 75 mg po once each day for 10 days. No prophylaxis is available for SARS.**

2. **Exposed workers should be instructed to be vigilant for the development of fever, cough, sore throat, runny/ stuffy nose, myalgia, headache, and diarrhea. Exposed workers will monitor themselves for fever (100°F or 37.8°C) and symptoms for 10 days after their last unprotected exposure to SARS and for 7 days after their last unprotected exposure to avian or swine influenza (see Appendix G).** If symptoms develop, exposed workers should immediately notify their supervisor, and their supervisor should notify Healthcare

Epidemiology and the Emergency Department.

3. Exposed workers should be actively monitored for symptoms prior to reporting for duty. (See Policy 3.9 Post Exposure Monitoring of UTMB Employees for an Emerging Infectious Disease [EID])

L. Management of Symptomatic Laboratory Workers with No Recognized Exposures
Laboratory workers who develop a fever or lower respiratory symptoms and who have no recognized exposure should immediately contact their supervisor. The supervisor should immediately contact Healthcare Epidemiology at 23192 or pager (409) 643-3133 after hours 24/7.

References

1. CDC Public Health Guidance for Community-Level Preparedness and Response to Severe Acute Respiratory Syndrome (SARS):

- Supplement F: Laboratory Guidance, Appendix F5: Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV
  www.cdc.gov/ncidod/sars/guidance/index.htm Accessed June 6, 2006.
- Supplement I: Infection Control in Healthcare, Home, and Community Settings, Section VIII: Infection Control for Laboratory and Pathology Procedures
  www.cdc.gov/ncidod/sars/guidance/index.htm Accessed June 6, 2006.

2. CDC. Interim CDC-NIH recommendation for raising the Biosafety level for laboratory work involving noncontemporary human influenza viruses. www.cdc.gov/flu/h2n2bsl3.htm. Accessed June 4, 2006.

Appendix A
Hematology

1. All EID specimens will be in a plastic bag with a yellow ABP sticker when arriving in the laboratory area.

2. When bag is opened and the specimen container is removed, all surfaces of the container should be wiped clean with a disinfectant.

3. After specimens are processed, decontaminate work surfaces and equipment. Use any EPA-registered hospital disinfectant. Follow manufacturer's recommendations for use-dilution (i.e., concentration), contact time, and care in handling.

4. Disposable hemacytometers will be used.

5. Hemacytometers will be loaded in a BSC with the operator wearing gloves and a fit tested N-95 mask.

6. The wash waste for the COAG machine will be picked up by Environmental Health and Safety weekly.

7. Trash receptacles for PPE and containers of alcohol gel for disinfecting hands before removing masks should be available near each automated instrument.

8. Maintenance will be done weekly on the XT 1800 with the operator wearing goggles, gown, gloves and a fit tested N-95 mask. Bleach at a 1.10 dilution will be run through the machine.

9. Weekly maintenance will also be performed on the XE 1200. Bleach at a 1:10 dilution will be run through the machine. The operator will wear goggles, gown, gloves and a fit tested N-95 mask.

10. Care will be taken in disposing of specimens and disposables. Double biohazard bagging will be done for increased safety to laboratory workers. Double bagging will be accomplished by having the person wearing PPE drop the bag containing contaminated trash into a clean bag held by a person wearing an N-95 mask and gloves.

11. If there are any questions or concerns about safety issues, please take them to your supervisor immediately.

12. Biohazardous waste will be removed by Environmental Services for decontamination and disposal.

Appendix B
Chemistry

1. All blood specimens should be spun in chemistry using sealed centrifuge rotors or sample cups. Sample cups or rotors should be loaded and unloaded in a BSC Class II in either the AFB laboratory or microbiology processing area. Personnel must wear a fit tested N-95 mask, gown and gloves during this process. Trash receptacles must be available inside or immediately adjacent to the BSC to avoid contaminating other areas of the laboratory. Pour-overs to secondary containers will be done inside a BSC.

2. Once the caps are removed, parafilm will be placed on the tubes for transport back to the chemistry laboratory.

3. A clinical laboratory employee wearing gloves and mask will stand next to the BSC holding a clean specimen bag to receive the container and original bag. He/she will seal the outer bag and remove the double-bagged specimen to a designated storage area. Then he/she will discard the gloves, wash hands, remove the mask and wash hands again.

4. Bleach should be run through the IRIS international remote imaging system weekly.

5. Store capped and parafilmed blood tubes in a biohazard bag at appropriate temperature until sent for autoclaving.

6. Open caps (even if it is not the original cap) under the BSC.

7. Blood samples (blood, serum, plasma) and urine samples should be handled using All Barrier Precautions which includes use of gloves, gown, goggles and an N-95 mask.

8. EID trash will be double bagged and a person wearing a mask and gloves will stand outside the door of the AFB laboratory with a clean bag to receive the trash. The person outside the door will then remove gloves, wash hands, remove the mask and then wash hands again. Disposable items such as slides and cuvettes used in testing EID specimens on the Vitros or Centaur instruments will be discarded immediately into puncture-resistant biohazardous waste containers and disposed of properly.

9. After specimens are processed, decontaminate work surfaces and equipment. Use any EPA-registered hospital disinfectant. Follow manufacturer's recommendations for use-dilution (i.e., concentration), contact time, and care in handling

Appendix C
Blood Bank

1. Tubes containing blood specimens will first be wiped with a disinfectant. Tubes containing blood specimens will be transported in a test tube rack and centrifuged using sealed centrifuge rotors or sample cups. Sample cups or rotors should be loaded and unloaded in the BSC located in the Transfusion Service, by personnel wearing gown, gloves, and a fit-tested N-95 mask.

2. Tubes will be opened in the BSC-Class II in the Transfusion Service.

3. All necessary equipment and trash receptacles should be located in the BSC or immediately adjacent to it to avoid contamination of other areas of the laboratory. A Blood Bank employee wearing gloves and an N-95 mask will stand next to the BSC holding a clean specimen bag to receive the specimen (double-bagged). After taking the double-bagged specimen to a clean area, hands will be washed, mask removed and hands washed again.

4. Samples <u>will not</u> be loaded on the Provue to determine ABO and Rh and to perform antibody screen.

5. ABO, Rh and antibody testing will be performed in the BSC located in the Transfusion Service laboratory.

6. Discarded waste should remain in bleach for one hour prior to emptying.

7. Rinse out disposal container after discarding the contents in the drain.

8. If the antibody screen is positive, determine specificity and perform antigen testing and extended crossmatch in the BSC in the Transfusion Service laboratory. The MTS centrifuge and incubator will be placed in the BSC during testing. The BSC should be turned on and allowed to run for at least 20 minutes before using.

9. EID trash will be double-bagged with the assistance of a person wearing an N-95 mask and gloves standing outside the door of the laboratory. After completion of the double-bagging, the person outside of the laboratory will wash hands, remove the mask and wash hands again.

10. Decontaminate all equipment and work surfaces with disinfectant. Run bleach through all cellwashers.

Appendix D
Microbiology

1. All specimens for culture with ABP stickers are delivered to the Receiving area in the microbiology laboratory. Serum samples for serology are delivered to Sample Management. Chemistry techs will process the samples if required. Otherwise, the serology techs will process samples in the virology BSC and use the aerosol safe cups in the centrifuge in serology or the AFB laboratory. All necessary equipment as well as trash receptacles should be located inside the BSC or immediately adjacent to the BSC to avoid contaminating other areas of the laboratory.

2. All culture specimens are plated in the BSC in the AFB laboratory by personnel wearing a gown, gloves and a fit-tested N-95 mask. All plates will be sealed with elastic tape. The original specimen container will be placed back into the original specimen bag. A microbiology employee wearing gloves and an N-95 mask will hold a clean bag out next to the BSC and the original specimen bag placed into it. After sealing the bag and removing the bag to a designated storage area, this person will wash their hands, take off the mask and wash their hands again.

3. All specimens are processed in the BSC in the AFB, virology or receiving laboratories by personnel wearing a gown, gloves and a fit-tested N-95 mask. This includes all culture plates, slides and biochemical tests. Blood culture bottles will be processed inside the BSC located in the receiving area. Viral EIA and DFA tests are processed in the BSC in either the AFB laboratory or the virology laboratory by personnel wearing a gown, gloves and a fit-tested N-95 mask.

4. Plates will be opened and biochemical tests performed by personnel wearing gown, gloves, and a fit-tested N-95 mask in the BSC in the AFB, virology or receiving laboratory.

5. If samples require cytocentrifugation, the Cytospin will be loaded and opened inside the BSC. The interior carrier will be opened inside the BSC.

7. After specimens are processed, decontaminate work surfaces and equipment. Use any EPA-registered hospital disinfectant. Follow manufacturer's recommendations for use-dilution (i.e., concentration), contact time, and care in handling.

8. Dispose of all disposable PPE in a clear plastic bag. Place this bag into another clear bag for removal.

9. EID trash should be double-bagged. A person wearing gloves and an N-95 mask will stand outside of the door to the AFB or Virology laboratory with a clean bag and hold the bag so that the contaminated bag held by the person in the laboratory can be dropped into the clean bag held outside the door.

Appendix E
Autopsy

1. EID patients will have a dedicated gurney and space in the cooler.
2. The body and body bag of EID patients will have yellow ABP stickers attached.
3. One autopsy room will be dedicated for autopsies on EID patients. Autopsies may be performed on other deceased patients without infections in other autopsy rooms at the same time that an autopsy is being performed on a patient with an EID.
4. A technical container will be available with all supplies for an EID autopsy in the dedicated EID autopsy room.
5. EPA hospital grade disinfectants will be stored in the Autopsy room.
6. The computer in the autopsy room will be covered during the case.
7. Faculty will be present at all times during the autopsy. A tech, PA or resident may also be present.
8. No other persons may be present during an autopsy on an EID patient.
9. The Stryker saw will not be used.
10. If the cranium must be opened, a special kit (autopsy head drape) will be used.
11. All specimens sent out of the room will be in formalin. The outer surface of the specimen container will be disinfected with a hospital-grade disinfectant.
12. The camera will be cleaned and passed out the door to a person wearing a gown and gloves. The person outside the room will again disinfect the camera, take off the gloves and gown taking care to avoid self contamination and then wash hands or apply an alcohol hand gel.
13. When removing PPE at the end of the autopsy, the outer pair of gloves will be taken off by turning them inside out and discarding them. Next remove goggles and /or face shield by grasping the ear pieces behind the goggles or head band behind the face shield and carefully and slowly moving them forward away from the face. Discard the goggles and/or face shield in the trash. Then remove the gown by slowly turning it inside out, and discard it in the trash. Remove the Kevlar gloves, and discard them in the trash. Remove the shoe covers and then the inner pair of gloves being careful to turn them inside out. Leave the autopsy suite, wash hands with an antimicrobial soap and water and then remove the mask by pulling the lower rubber band over the head and allowing it to dangle beneath the chin. Next grasp the upper rubber band behind the mask and slowly lift up and move the mask in a straight line away from the face. Discard the mask and wash hands with an antimicrobial soap and water or apply an alcohol hand rub.
14. Everything will be discarded including the Kevlar gloves.

Appendix F
Surgical Pathology

1. All EID specimens received in formalin must fix for at least 1 hour/mm maximal thickness prior to processing.

2. Fresh tissue specimens are to be accessioned and transported in the presence of an attending Pathologist to Microbiology where appropriate samples are obtained within a Biological Safety Cabinet. Fresh tissue specimens will be transported in a closed sterile container. The outer surface of the specimen container will be disinfected with a hospital grade disinfectant and placed in a plastic ziplock bag.

3. The attending pathologist or designees from surgical pathology should wear a gown, goggles, gloves and an N-95 mask while performing gross sectioning such that the tissue may be allowed to fix in formalin.

4. Once the tissues have been allowed time for formalin fixation (in general, 1mm/hour thickness), the specimen may be prosected for routine histological processing.

5. Empty specimen containers will be discarded in a biohazard bag.

6. The grossing bay and all dissecting utensils and material must also be decontaminated by thorough cleaning with a hospital grade disinfectant.

7. After specimens are processed, decontaminate work surfaces and equipment. Use any EPA-registered hospital disinfectant. Follow manufacturer's recommendations for use-dilution (i.e., concentration), contact time, and care in handling.

Appendix G

Employee Monitoring Form
for an Emerging Infectious Diseases (EIDs)

Name_____

Check and record temperature twice daily at 0800 and 2000 hours (8 AM and 8 PM). Please check Yes or No for each of the symptoms listed on the table below every day. If you develop fever (100° F or 37.8° C) and ≥ 2 symptoms in the list in the table below, if at work, notify your supervisor, don a surgical mask, practice hand hygiene and leave the worksite; if at home, remain at home and notify your supervisor.

	DATE	TEMPERATURE		SYMPTOM CHECK LIST							
		8 AM	8 PM	COUGH	SORE THROAT	RUNNY NOSE	MUSCLE ACHES	CHILLS	HEAD ACHE	VOMITING	DIARHEA
1				YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯
2				YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯
3				YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯
4				YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯
5				YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯
6				YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯
7				YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯
8				YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯
9				YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯
10				YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯	YES ◯ NO ◯