CHAPTER

16

THE INFINITI™ SYSTEM- AN AUTOMATED MULTIPLEXING MICROARRAY PLATFORM FOR CLINICAL LABORATORIES

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16.1. Introduction

Genetic analyses have progressed in a relatively short time from the traditional 'one gene at a time' approaches to detailed surveys of complete genomes. Professor Ed Southern is globally recognized for his key insight over 25 years ago that labeled nucleic acid molecules could be used to interrogate genetic material to determine Deoxyribonucleic acid (DNA) sequences[1,2]. The Southern blot technique and its derivatives have created extensive research programs in many different fields of biology, diagnostics and medicine. DNA microarrays permit the simultaneous analysis of many nucleic acids in parallel. This technology is providing a new landscape on the inner workings of cells and gene regulatory networks by taking a snapshot of the transcriptional state of a cell at a given point in time. DNA microarrays are currently the preferred screening method for high throughput transcriptional profiling. Microarrays have proven of great value in genomics research[3,4,5] and have been widely utilized in drug target validation and discovery efforts[6]. Microarrays are currently poised to enter the clinical arena and provide improved genetic testing thereby facilitating the processes of disease diagnosis, pharmacogenomics, and toxicogenomics[7,8].

Currently, the methods employed for genetic testing are labor intensive and highly complex, and require the simultaneous analysis of multiple nucleic acid markers. Microarray technology is without doubt the most practical approach to multiplex and analyze biomolecular markers. However, the use of microarray technology remains practically non-existent in the clinical environment due to the paucity of instrumentation that provides integrated automation with result analysis. The emergence and success of microarrays in the clinical laboratory is dependent on their ability to adapt to the rigorous environment of routine usage whilst providing high quality, reproducible and robust results. The clinical environment stretches the limits of this technology as it measures performance criteria in a different manner to the research environment. One key difference from an economic standpoint is that the cost per reportable result is more important than the cost per spot. Other important factors are the requirements for automation from sample processing to end result, reproducibility, accuracy of results and the ability to process large volumes of tests under strict regulatory guidelines and compliances.

In the clinical laboratory setting, where very large numbers of patient samples are processed, an automated platform that permits multiplexed assays, provides cost containment and increased workflow efficiency is highly desirable. Commercial instruments are typically discrete analyzers that perform specific DNA analysis tasks and are in essence "islands of automation". This results in elevated costs per reportable result, the requirement for method specific instrumentation, high labor costs requiring skilled operators, multiple workstations,

high error rates and poor reproducibility. The emergence of completely automated clinical microarray platforms has been a slow process due to the enormous challenge in integrating multiple components such as the detection system, robotics, sample and reagent handling, operating software and result analysis.

In this chapter, we discuss the INFINITI™ system that we have developed for the clinical laboratory and present experimental applications. We have integrated the discrete processes of sample handling, reagent management, hybridization and detection for the analyses of DNA and proteins into a totally self-contained multiplexing platform. The INFINITI™ System offers complete "walk-away" automation by employing four key components:

- **BioFilmChip**™ microarray
- **INFINITI**™ Analyzer with sample to result automation
- **Qmatic**™ operating software with applications interface
- **Intellipac**™ reagent management module

16.2. The BioFilmChip™ Microarray

Microarrays have traditionally been based on microscopic glass slides and have been widely employed in research. Glass slides are not practical for routine clinical laboratory application because they are open and reagent volumes are difficult to maintain in a consistent manner. Furthermore they are susceptible to cross contamination and evaporation. To resolve these issues, we have developed a novel film-based microarray, The BioFilmChip™, which consists of multiple layers of porous hydrogel matrices about 8-10 µm thick on a polyester solid support (Figure 16-1). This provides an aqueous microwell and a three dimensional environment that is highly compatible with biological materials. The second layer incorporates a proprietary composition for removing intrinsic fluorescence, which is essential for improving assay sensitivity and for eliminating potential artifacts due to fluorescent "hot spots". The top layer is designed for immobilization of biological molecules such as oligonucleotide, antibodies or antigens. The biological molecules can be coupled covalently using gluteraldehyde, imidoester and epoxides or non-covalently using strepatavidin-biotin interaction[9,10].

The chip is relatively inexpensive to fabricate and scale-up is facilitated by the use of conventional web coaters such as those used in the photography industry. Once fabricated, the chips are housed in a magazine (Figure 16-2). The BioFilmChip™ microarray developed for clinical use will rarely need a density of greater than 100 spots per chip, yet it is possible to print 1024 spots on a particular film chip. For gene expression and/or protein expression analyses,

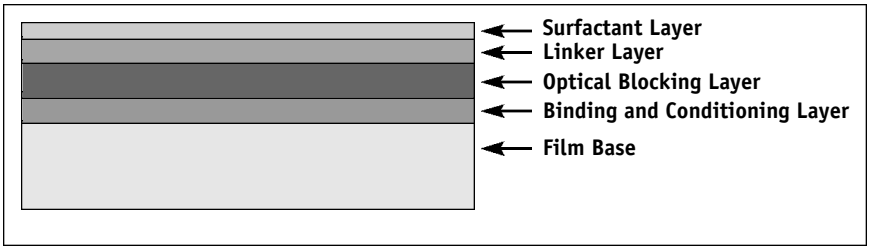


Figure 16-1: Schematic of the BioFilmChip™ Microarray with multiple layers. The chip consists of multiple layers of porous hydrogel matrices about 8-10 µm thick on a polyester solid support.

Figure 16-2: BioFilmChip™ (left) and BioFilmChip™ Magazine for housing 12 chips (right).

which require thousands of spots, multiple chips can be utilized. The BioFilmChip™ microarray can be fabricated using both contact and non-contact printing[11,12]. Replicating, quill or slit pins, in addition to jetting methodologies (piezoelectric, ink jet and bubble jet) can be used.

16.3. The INFINITI™ Analyzer

The INFINITI™ Analyzer (Figure 16-3) is an automated, continuous flow, microarray platform that integrates all the discrete processes of sample han-

dling, reagent management, hybridization and detection for the analyses of DNA and proteins in a totally self-contained system. A confocal microscope has been integrated into the analyzer with two lasers (red and green). In addition, a thermal stringency station and a thermo cycler for denaturing nucleic acids for primer extension studies or hybridization reactions in solution have been incorporated. The system is designed to operate in a continuous random access mode. To avoid contamination of samples or reagents, disposable pipette tips are used for each step in the assay. This eliminates the use of pumps, plumbing or tubing and biohazardous liquid waste.

Figure 16-3: The bench top INFINITI™ Analyzer.

16.3.1. Detection System

The INFINITI System is integrated with a confocal microscope controlled by the system software scheduler. It has two lasers: a laser with an excitation wavelength of 632.8nm and a second laser with an excitation wavelength of 543.5nm. Fluorescence emissions are measured at 650nm and 560nm, respectively. For fluorescence signal measurement, a high sensitivity photo multiplier tube is incorporated into the system. The microarray is automatically inserted into the scanner, which possesses X, Y Z motion capability and 0.5 micron step resolution. It uses a digital camera for focusing and for registration. The dynamic range exceeds four orders of magnitude and scanning is performed in one to three minutes, depending on the assay protocol. A schematic of the confocal microscope is depicted in Figure 16-4.

The operator has the option to use commercially available software for data normalization for single nucleotide polymorphism (SNP) scoring, gene expression and protein expression analysis, statistical, pattern-recognition, and data reduction software such as clustering algorithms and curve fitting methods with the INFINITI™ Analyzer. A number of approaches for data normalization and transformation have been utilized such as linear regression analysis, log centering and Chen's ratio statistics[13,14]. The INFINITI™ Analyzer offers a number of data analysis and data reduction capabilities. For example, a curve fitting method based on four parameter log-logistic transformation is incorporated for immunoassay studies. Additionally, Levey Jennings charts are available for performing internal quality control.

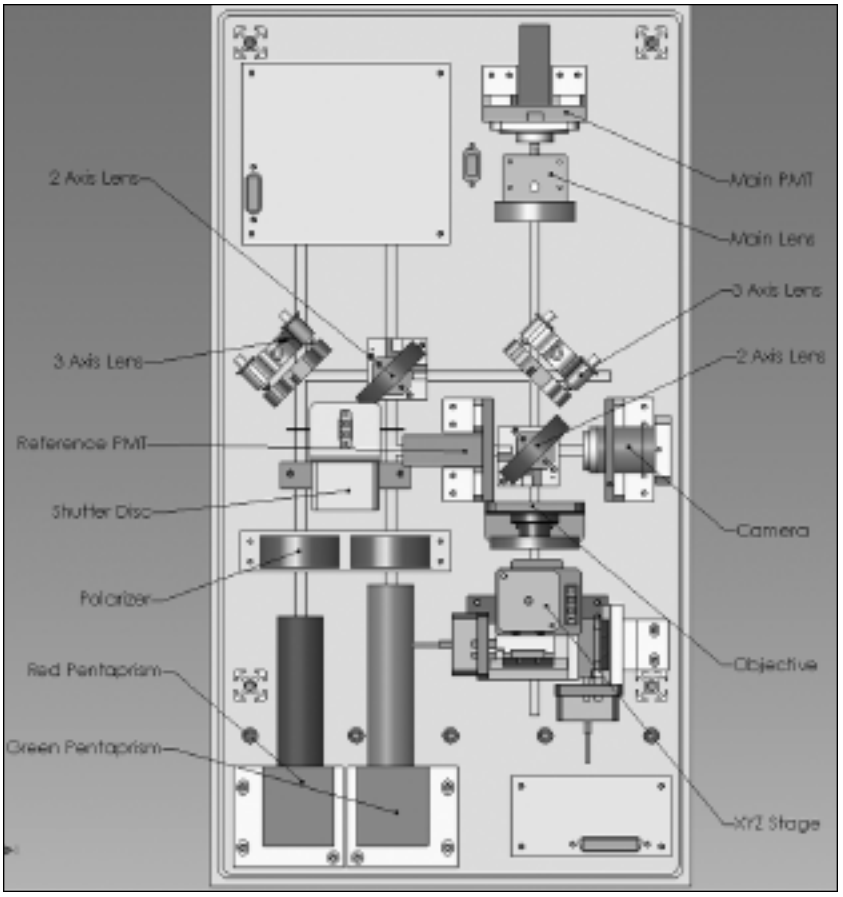


Figure 16-4: A view of the confocal microscope, two lasers and digital camera.

16.4. Qmatic™ Operating Software

The Qmatic™ operating software provides unprecedented flexibility and simplicity for performing both genomics and proteomics analyses. The multi-tasking software manages the complex tasks performed by the INFINITI™ Analyzer. It controls all the operations of the system such as assay protocol, robotics (aspiration and dispensing), fluorescence signal measurement, data analysis, data handling and generation of report. It identifies samples and queries the Laboratory Information Management System (LIMS) for assays to be performed and prompts the operator to load the assay specific reagents such as the BioFilmChip™ Microarray and Intellipac™ reagent modules. In addition, it also calculates and monitors the amount of reagents, the number of pipette tips and wash solution required to complete the test(s). For research applications, the software allows a researcher to develop custom assays efficiently using its Generalized Assay Protocol editor (GAP).

16.5. Intellipac™ Reagent Management Module

A critical aspect of this analytical system is the reagent management module, which acts as a communication link (Figure 16-5). It has eight reservoirs to house the appropriate reagents for a given test and has an integrated 128 K memory chip. This serves to simplify the work process for the operator and records all data electronically. The assay protocol for a specific test resides in

Figure 16-5: Intellipac™ Reagent Management Module. A critical aspect of Infiniti system is the reagent management module, which stores pertinent data such as the expiration date of reagents, the volume of reagents used, the time of use and the particular operation performed.

this chip and is uploaded to the analyzer upon request. Pertinent data such as expiration date of reagents, volume of reagents used, time of use and operation information are all stored in memory. The reagent has a movable lid that is opened and closed automatically by the analyzer to control evaporation and minimize potential reagent contamination. Real time technical support via the Internet is made available through the Intellipac™.

16.5.1. "Walk Away Automation"

To perform a test, the operator generates a work list, loads the sample in a microtiter plate, and the appropriate microarray magazines and reagents. The system manages the workflow and processes the entire test seamlessly without manual intervention. The turnaround time of an assay is dependent on the type of assay being run. The time to first results using hybridization methodology and immunoassay are less than one hour and three hours, respectively. The analyzer can store data, perform data analysis and data reduction. The 80 gigabyte hard drive facilitates storage, formatting and personalization of the results to particular laboratory requirements.

The "open architecture" design enables adaptation of multiple methodologies such as hybridization, primer extension and cleavase assays, and both competitive and sandwich immunoassays. Researchers have the flexibility to develop test protocols for their own custom applications. The system can process 24 microarrays simultaneously.

To perform a hybridization assay on board the analyzer, appropriate capture probes are immobilized on the microarray and are loaded in magazines on to the analyzer (Figure 16-3). Sample (target) and appropriate reporter probes are added to the microarray from the Intellipac™. The target hybridizes with the capture and reporter probes. Following hybridization, the fluorescence signal is measured at the detection station. A schematic representation of the hybridization assay workflow is depicted in figure 16-6.

To perform a single nucleotide primer extension assay on board the analyzer, appropriate capture probes are immobilized on the microarray and loaded in magazines on to the analyzer (Figure 16-3). Sample (target) is added to the microarray and hybridized. Following stringency washing, fluorescent-labeled nucleotide analogs (chain terminators) and DNA polymerase are added to the microarray from the Intellipac. Following the extension reaction the chip is washed and the fluorescence signal is measured at the detection station. A schematic representation of the SNP assay workflow is depicted in Figure 16-7.

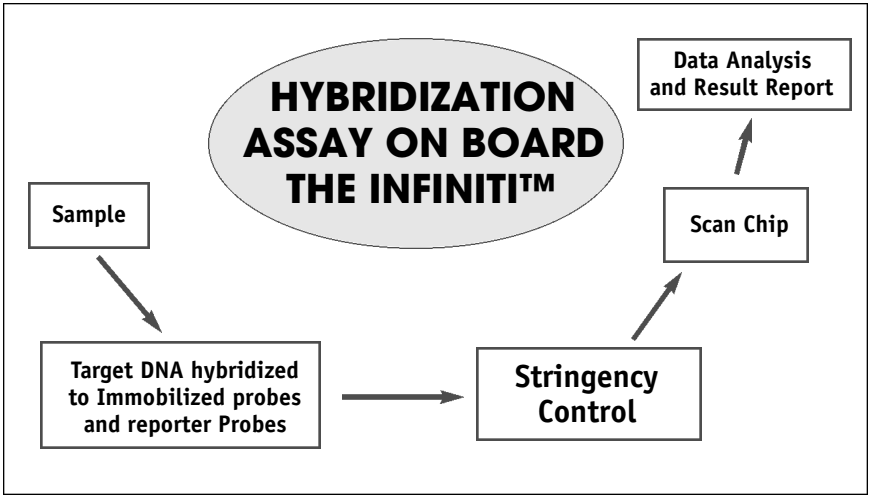


Figure 16-6: Schematic representation of the hybridization assay.

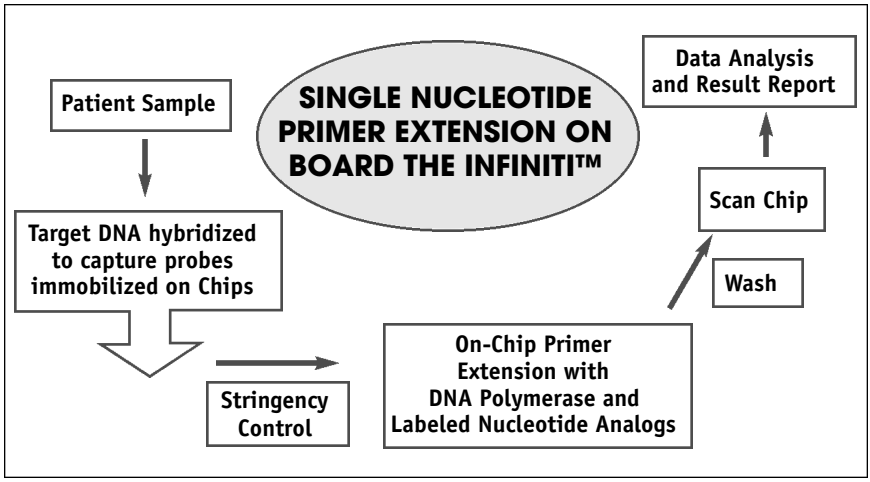
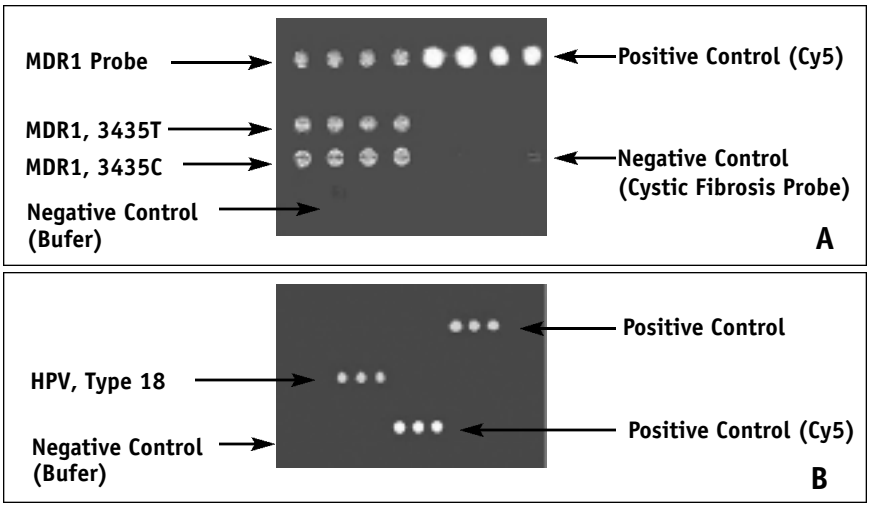


Figure 16-7: Schematic representation of the primer extension assay.

16.6. Experimental and Results of Example Applications using the INFINITI™ System

Oligonucleotides (25-33 mers) were synthesized by IDT (Coralville, IA, USA) and were spotted on the BioFilmChipTM using a custom arrayer. The distance between spots was 400 µm. The BioFilmChips™ were washed to remove the unbound capture oligonucleotides and stored desiccated at 4-8°C. Briefly, DNA was extracted using the QIAamp DNA Blood Mini Kit (QIAGEN Valencia, CA, USA). PCR was performed (30-40 cycles) using a Flexigene 96 well PCR cycler (Techne, Cambridge, UK) to amplify Human papillomavirus (HPV), Connexin26 (CX 26), Cystic Fibrosis (CF), Multidrug resistance 1 (MDR 1), Factor V, Factor II (G20210A)and Factor V (G1691A) and MTHFR 677 (C677T) and 1298 (A1298C). For the CX26, CF, MDR1, Factor II, Factor V and MTHFR assays, 25-50 ng of the isolated genomic DNA was used to generate the target products in 25 µL of PCR reaction. The samples (PCR amplicons) and the Intellipac™ containing Cy5 labeled dCTP, dGTP, dTTP, dATP, shrimp alkaline phosphatase (SAP), and exonuclease I reagents were loaded on to the INFINITI™ Analyzer and treated with shrimp alkaline phosphatase and exonclease to inactivate the excess primers and dNTPs. The treated amplicons (5 µL) were used as templates for multiplexing Allele Specific Primer Extension (ASPE). The extension products were hybridized at 39°C for 30 minutes to the immobilized capture probes on the BioFilmChip™ Microarray and scanned. The resultant images are depicted in Figure 16-8.

For the HPV assay, 1-3 µL of the purified DNA samples were amplified (40 cycles) and 1-3 µL of the first round PCR products were used as template in a second PCR reaction with fluorescent-labeled nucleotides. PCR products (25 µL)



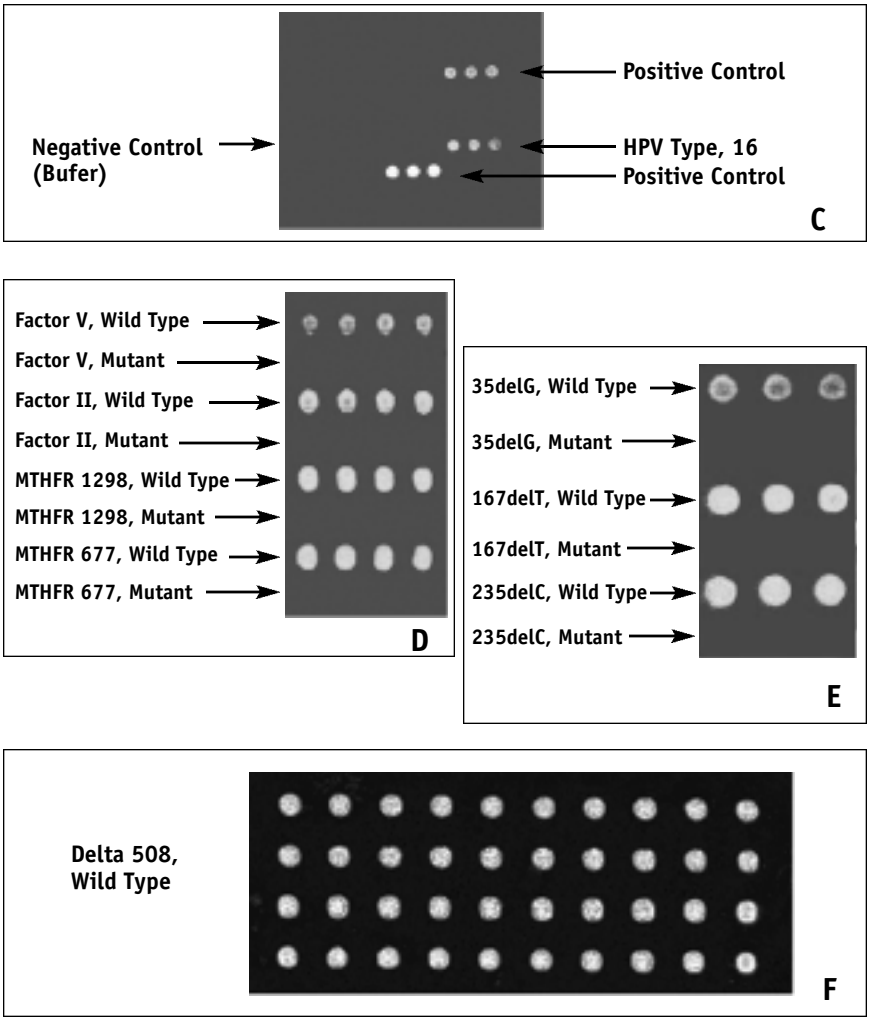


Figure 16-8: Applications performed using the INFINITI™ System.
A: MDR1 assay for SNP at position 3435.
B: Hybridization assay for Human papillomavirus (HPV), Type 18.
C: Hybridization assay for Human papillomavirus (HPV), Type 16.
D: Multiplexed assay for Factor V, Factor II and 5,10-methylenetetrahydrofolate reductase (MTHFR) based on Allele Specific Primer Extension.
E: Multiplexed assay for Connexin26 based on Allele Specific Primer Extension.
F: Assay for Cystic Fibrosis (delta F508) based on Single Nucleotide Primer Extension. The image depicts the wild type genotype.

were mixed with an equal volume of hybridization buffer and dispensed on to the BioFilmChip™ for hybridization. Following hybridization, chips were washed under stringent conditions and scanned (Figure 16-8C).

16.7. Conclusion

The INFINITI™ system is a self-contained, user-friendly multiplexing microarray platform with an "open architecture" that has been primarily designed to meet the needs of clinical laboratories and researchers working in the fields of genomics and proteomics. The flexibility of the system for performing multiple methodologies for molecular diagnostics has been demonstrated. Application of the INFINITI™ system in proteomics is covered in detail elsewhere[15].

The wide acceptance of microarrays for routine genetic testing will require that the technology meet the requirements of the clinical laboratory. Clinical DNA and RNA reference standards, and data processing algorithms are yet to be established. However, such materials may be available in the near future from institutes such as the National Institute for Biological Standards and Control (NIBSC). In addition, validation and quality control are important throughout the whole process, including fabrication of chips, sample preparation (nucleic acid extraction and amplification), detection, and analysis. The INFINITI™ system is a multiplexing platform that enables a laboratory to perform genomic and proteomic analyses on routine basis. The versatility of the platform demonstrates its potential for use in research and drug discovery applications. Initial test applications on the INFINITI™ System include routinely performed "Home Brew " tests such as cystic fibrosis, multiplexed thrombophilia panel (Factor II, Factor V and MTHFR), cytochrome P450 for drug metabolism and TH1/TH2 immune response panel. Future applications will include a wide spectrum of tests, including apoptosis markers and infectious disease tests.

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