PALEOPATHOLOGY IN CHAGAS DISEASE

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REDUVIID (KISSING BUG-CHIRIMACHA)

THERE ARE MANY SPECIES OF TRIATOMINE BUGS AND NUMEROUS MAMMALIAN HOSTS FROM NORTHERN ARGENTINA AND CHILE TO NORTHERN CALIFORNIA. LOCALLY ACQUIRED INFECTIONS IN HUMANS ARE GENERALLY VERY LIMITED, AND ONLY PRODUCED BY BUGS THAT HAVE ADAPTED TO COLONIZING HUMAN DWELLINGS. THIS IS BASICALLY 5 SPECIES. THESE INSECTS DEPOSIT THEIR FECES CONTAINING THE T. CRUZI AND THEY ENTER THE BODY BY SCRATCHING THE BITE SITE OR VIA THE MOUTH OR THE EYES. THEY MAY ALSO BE TRANSMITTED BY BLOOD OR ORGAN TRANSPLANTS.
1885 BROUGHT TO PERU BY CHILEAN SOLDIERS IN WAR OF THE PACIFIC
1907 MINAS GERAIS, BRASIL DISCOVERY
1950 FOUND IN AREQUIPA/MOQUEGUA PERU
1990 BROUGHT TO JAEN, NORTHERN PERU BY INFECTED DOCTOR FROM AREQUIPA
2000 FOUND ALL OVER PERU

CARLOS CHAGAS 1879-1974
• GRADUATED MEDICAL SCHOOL, RIO, BRASIL 1903
• MALARIA CONTROL OFFICER, LASANSE, 1907
• LOCAL INHABITANTS COMPLAIN THEY WERE BITTEN BY A BLOOD-SUCKING BUG AT NIGHT.
• CHAGAS COLLECTED BUGS AND FOUND PROTOZOAN FLAGELLATES IN THEIR FECES.
• HE FOUND THE SAME CIRCULATING TRYPANOSOMES IN THE BLOOD OF CHILDREN WITH ACUTE FEBRILE ILLNESS.
• THIS IS THE FIRST TIME THE VECTOR OF A DISEASE WAS FOUND BEFORE THE DISEASE.

ACUTE PHASE CHAGAS DISEASE
USUALLY CHILDREN – FATALITY RATE 10%
INCUBATION PERIOD - 2 WEEKS/SEVERAL MONTHS
MAY BE ASYMPTOMATIC
MAY BE LESION AT PORTAL OF ENTRY – CHAGOMA
FEVER – EDEMA, RASH, VOMITING
LYMPHADENOPATHY, HEPATOMEGALY
INFANTS – MULTIPLE SKIN CHAGOMAS
HEPATOMEGALY COMMON
CONVULSIONS, TREMOR, WEAK REFLEXES

CHRONIC CHAGAS DISEASE
HEART – 30% OF SURVIVORS ABNORMAL ECG
RIGHT BUNDLE BLOCK
AV CONDUCTION ABNORMALITIES
ARRHYTHMIA
CHEST PAIN, EDEMA, DIZZINESS
MEGACARDIA – EMBOLISM, SUDDEN DEATH
GASTROINTESTINAL – VARIES IN GEOGRAPHIC LOCALS
MEGAESOPHAGUS
MEGAcolon
INFLAMMATION OF MUSCULARIS LAYER
LOSS OF NERVE CELLS FROM AUERBACH’S PLEXIS
LOSS OF PERISTALSIS PRODUCES DEATH FROM FECAL IMPACTION

DOCTORAL THESIS – DR. URIEL GARCIA, 1951
5 CASES IN CHILDREN IN MOQUEGUA, PERU.
THEY HAD ROMANA’S SIGN OF ACUTE DISEASE
STERILE CHIRIMACHAS BITE CHILDREN
BUGS FECE HAD PROTOZOAN FLAGELLATES.
<table>
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FORTY FIVE BODIES RECOVERED IN ILO, PERU, 600 BEFORE PRESENT ABOUT HALF WITH SOFT TISSUE

HARRIS LINES OF ARRESTED GROWTH:
NEGATIVE 17
POSITIVE 28

PNEUMONIA 5
GHON COMPLEX 2
RECENT DELIVERY 3
CHAGAS DISEASE 1
ARTIFICIAL MUMMIFIED 10
WOMEN 6
MEN 2
CHILDREN 2

TARAPACA 22 AUTOPSIES-
WANKARANI CULTURE-500BC

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50% 6.9% 1.9%

MUMMIES WITH MEGACOLON HAD FECAL OBSTRUCTION WITH AS MUCH AS 3 KILOS OF DRIED FECES. FIVE HUNDRED MUMMIES FROM THE AZAPA VALLEY HAD ONLY ONE WITH MEGACOLON WITH FECES.
SEROLOGICAL SURVEYS IN LATIN AMERICA REVEALED:

• 18 MILLION CARRIED T. CRUZI

• 90 MILLION WERE EXPOSED TO INFECTION

• 500,000 ARE INFECTED IN USA
Chagas Disease Today

Chagas disease is an infection caused by the flagellated protozoan parasite Trypanosoma cruzi. This disease is also referred to as American trypanosomiasis. Chagas disease, found only in the American Hemisphere, has a wide distribution in Central and South America. It is endemic in 21 countries. The overall prevalence of human T. cruzi infection is 16-18 million and 120 million, or 20%, of the inhabitants of Latin America, are at risk of acquiring the infection. T. cruzi infections are primarily transmitted to humans by infected bugs, also known as kissing bugs, that live in cracks and holes of the substandard housing commonly found in South and Central America. Common tritamine vector species for trypanosomiasis belong to the genera Triatoma, Rhodnius, and Panstrongylus. This infection can also be transmitted by an unsterilized blood transfusion. The prevalence of infected blood in blood banks in selected cities of the South American continent is documented to be above 1%. In some, but understandably not all, countries in South and Central America, 100% of blood donors are screened. Between 1990 and 1995, the prevalence of infected blood in blood banks in selected cities of South America ranged from 1% in São Paulo, Brazil to 53.5% in Santa Cruz, Bolivia, a percentage far higher than that of hepatitis B and C or HIV infection. As a consequence, infection rates among blood recipients vary from 0-1.2% in Argentina, Brazil, Chile and Uruguay, to as high as 24.6% in Bolivia. The infectivity risk, defined as the likelihood of being infected when receiving an infected transfusion unit, has been estimated at 2%. Other methods of T. cruzi transmission include organ transplantation and congenital infection. However, infection on the effects of T. cruzi infection on pregnancy occur despite the fact it is an endemic area, such infections affect 1% in 51% of pregnant women of which 2% in 10% transmit the parasite to their foetuses. Chagas’ disease was present in 17.22% of pregnant women undergoing hysterectomy in an Argentinean hospital. It has also been reported in cardiac transplant patients in the USA and other countries.

Chagas disease occurs in two stages. The first, acute stage appears shortly after the infection; however, acute symptoms only occur in about 1% of cases. Instead, the majority of infected persons are asymptomatic, with an indeterminate number eventually developing symptoms of the second, chronic stage after a silent period that may last several years. The lesions of the chronic phase inversely affect several internal organs, namely the heart, the oesophagus, and the colon; they can also affect the peripheral nervous system. After a several year asymptomatic period, 25% of those infected develop cardiac symptoms which may lead to sudden death, 5% develop digestive damage, mainly megacolon, and 5% of these will present with peripheral nervous involvement. In 20%, subacute damage is observed with either asymptomatic or associated with fever and other mild symptoms, one of those being the chagoma, flat lump which is surrounded by a red area or skin lesion, usually at the bite site where insect feces was rubbed into the eye. The other nonspecific symptoms seen in acute Chagas disease may include fatigue, fever, anorexia or fever, weakness, or vomiting. Sometimes, a rash, loss of appetite, diarrhea, and vomiting occur. In general, symptoms last for 4-8 weeks and then resolve, even without treatment. However, occasionally, life-threatening myocarditis or megacolonitis can occur during the acute phase, particularly in young children and immunocompromised patients. After years to decades of subclinical infections, 10-50% of infected persons develop chronic Chagas disease, which is characterized by potentially lethal cardiac arrhythmia or megacolonitis (i.e., megacolon, megacolonitis, and megacolonitis). Even persons who remain asymptomatic probably are infected and infection for life, with low levels of the parasite in their blood and other tissues. In persons who are immune-compromised, including persons with HIV/AIDS, Chagas disease can be a fatal disease; megacolonitis occurs in 70% of the patients who present with artificial or modified chagomas lesions which are often not responsive to drug treatment. Not everyone develops the chronic symptoms of Chagas disease.

In most cases treatment of symptoms is all that is possible. Currently available medications are used to reduce the duration and severity of an acute infection, but are only 50% effective, or less, in eliminating the organism.

Cardiac disease is managed with antiarrhythmics and medications. Esophageal complications require either endoscopic or surgical interventions to improve esophageal emptying, procedures similar to those used to treat the disorder known as achalasia. Conduction is treated by increasing their speed and the ratio healing (tricaglublina) 70% removal of the dissoxidated fat. Two drugs, isradipine and bumetanide, are capable of curing at least 50% of refractory megacolonitis. Being active in the acute and short-term (up to a few years) chronic phase. Viniti (1995) showed that the treatment of Chagas Disease in the indeterminate phase can prevent the development of the chronic disease. Fergus (1993) compared treatment with bumetanide versus observation of patients with another cardiac or asymptomatic disease and concluded that treatment is more effective in stopping or preventing disease progression (5% versus 14% in non-treated group).

Recently, several double-blind randomized trials showed that bumetanide may be useful for early stage disease in children under 12 years of age. Further studies with long-term follow-up will be necessary to determine the value of generalizing the use of the above medications. Infrequently, patients with Chagas disease regardless of age and disease stage. Regardless, the ideal treatment for Chagas disease has yet to be found. Although attempts to communicate animals have not been complete failures, current results are not adequate to put forth hope of a vaccine for use in man within the foreseeable future.

The burden of Chagas Disease

The medical and social impact of Chagas disease is high. It is estimated that about 752,000 working years are lost annually due to premature deaths caused by the disease in the seven countries of South America, a number that corresponds to 1.26% of all USYD of the world's population. The most important achievements of the program include a 72% reduction in the incidence of human infection in children and young adults in the countries of the Initiative of the Southern Cone. In 1997, Uruguay was certified free of vectorial and transfusional transmission of Chagas disease. In 1999, Chile was certified free of vectorial and transfusional transmission of Chagas disease. In 2000, the ten countries with the highest rates of transmission in Brazil were certified free of vectorial and transfusional transmission of Chagas disease.

Priorities and strategic emphasis for WHO/TDR-supported research on Chagas disease.

While elimination of the disease as a public health problem (through interruption of transmission) is a reasonable goal, eradication is not, due to the zoonotic nature of the disease.

The major problems and challenges are:

- Sustainability of vector control strategies and policies in the countries of the Southern Cone.
- Implementation of specific disease transmission control strategies in those countries where, besides domiciled sylvatic, sylvatic vectors also play an important role.
- Implementation of suitable diagnostic programs and treatment strategies and policies.
- Definition of a long-term research agenda for the future, aiming at the development of new prevention and control tools, such as efficient vaccines and safe drugs.

Research activities needed

- Precise epidemiological information about the magnitude of the morbidity and mortality associated with T. cruzi. Incidence of infections in young age groups.
- Discovery and development of new chemotherapeutic and diagnostic tools through creation of basic and clinical research networks and partnerships with the private sector.
- Susceptibility and resistance to insecticides, and other issues relevant to vector control.
- Sustainability of activities relevant for control of transmission based on blood transfusion.
- Technical and policy frameworks, for getting research into action in elimination programmes in Central America and the Andean countries. Also getting research into action in the management of congenital and indeterminate Chagas disease, is needed, as are harmonized guidelines relative to case definitions, management and treatment.
Our Paleopathological studies on human Andean mummies began in 1971 at the Museum of Sea, Southern Peru and later in 1973 at the Museum of Arica, Northern Chile. Archaeological materials and mummies from vandalized cemeteries were recovered by nationally known archeologists. The mummies were radiographed and autopsied. Samples of organs were taken for C14 and pathological diagnoses. The tissues were rehydrated in Ruffer’s solution, paraffin blocked and stained with the usual techniques performed in a modern Pathology Laboratory. Paleopathological features were found in the mummies of both geographic areas consistent with tuberculosis in soft and bone tissues. The soft tissues consisted of Ghon complex, cavitary pulmonary disease, miliary disease and in bones, Pott’s disease. Many tissues had visible acid-fast mycobacteria. García Frías had reported several cases of tuberculosis with bacilli in Peru in 1940, published in “Actualidad Médica Peruana” but at that time C14 was not available although acid-fast organisms were found in sections.

We reported the first case of tuberculosis in Pre-Columbian America in 1973 in the American Review of Respiratory Disease with stained acid-fast organisms in tissues and dated the mummy with C14. The report was from a mummy bundle excavated with all of its burial goods from the province of Nazca, Peru. The mummy, an 8-year-old boy, was found seated on an adobe seat with a thick cushion. Carvings dated the mummy around 700 A.D. The position of the legs is commonly seen in persons who are paralyzed in the lower limbs and unable to walk. The main findings in the gross examination were severe sclerosis in the lumbar area. Radiographs showed Pott’s disease with a right psoas abscess. During the autopsy, small nodules were seen in the lungs, pleura, liver and kidneys. Tissue sections from the organs were rehydrated and stained. Ziehl-Nielsen stains confirmed the gross diagnosis revealing many clumps of acid-fast bacilli in these lesions; mainly in the lungs and kidneys. The disease process in this case can be reconstructed by an X-ray of the tibia, which revealed Harris lines starting at about 3 years of age. The boy developed a chronic stage of this disease with secondary Pott’s disease. The terminal event was a re-infection with a hematogenous spread producing extensive pulmonary disease. In total, we reported 12 cases showing healed calcified Ghon complexes and primary active cases and reinfection cavitary disease of soft tissue and bone that had the pathological findings of tuberculosis. Acid-fast bacilli were found in only two of them.

The Medical College of Virginia (MCV), in Richmond, Virginia, had a 200-bed sanatorium that closed in 1967. Two important studies of tuberculosis were performed while the sanatorium was operating. The first one was comparing the outcome of patients from the MCV sanatorium who received anti-tuberculosis therapy, with another sanatorium in Farmville, VA, where the patients did not receive anti-tuberculosis therapy. Farmville had 1,841 patients treated between the years 1936 and 1945. The Medical College of Virginia sanatorium in Richmond had 2,205 patients treated between 1936 and 1967 with anti-tuberculosis drugs. The two Sanatoriums had similar clinical classifications of patients with tuberculosis as minimal, moderate and far advanced. The results showed that in the later years, the improvement rate of patients treated with antibiotics almost doubled. The gender incidence had little difference in both areas, but was slightly more common in males than females in the later years. The percentage of extra-pulmonary disease with pulmonary disease was reduced by half, (47% to 24%) in the anti-tuberculosis drug therapy group. It is interesting that in both groups the number of patients with extra-pulmonary disease per patient was the same, about 80%, 17% and 3% from one, two or three systems, respectively. The distribution of the systems in the two groups, however, was different. The patients who were untreated with antibiotics had extra pulmonary lesions more commonly in the larynx, gastro-intestinal tract and male reproductive system, but antibiotic treated patients had lesions more common in the urinary, central nervous and skeletal systems.

The second important study undertaken at the MCV sanatorium was related to the increased resistance of Mycobacterium tuberculosis to drug therapy seen shortly after the 3 drugs: Para-aminosalicylic acid, Isoniazid and Streptomycin were introduced in the treatment. Four hundred eighty two black, tuberculosis patients were followed after being admitted. Their organisms were tested for sensitivity to streptomycin, isoniazid and para-aminosalicylic acid. They were classified on the basis of their histories as new or old patients, depending on whether or not they had prior anti-tuberculosis therapy. Forty-four percent of the new patients and 67% of the old patients had organisms resistant to one or more of the three antituberculosis agents used. The patient’s rate of recovery decreased as the resistance of the organism increased. Tuberculosis patients that previously received anti-tuberculosis therapy developed chronic disease or died of their tuberculosis 3½ times more frequently than new patients without any prior anti-tuberculosis therapy.

References
Tuberculosis
New Facts about an Old Disease

Antonino Catanzaro, M.D.
Professor of Medicine
UC San Diego

Definition

- Tuberculosis was known as a specific disease by ancient cultures
- The disease was called by names like
  - consumption
  - ptysis
- The name tuberculosis was introduced when tiny nodules or tubercles were noted on examination of diseased lungs

Robert Koch

- March 24, 1892 announced his discovery that TB is caused by an infection caused by a microorganism
- Named the organism *Mycobacterium tuberculosis*
- Developed tuberculin and injected it subcutaneously

Pathogenesis of TB to Bug: *Mycobacterium tuberculosis*

- 1 or 2 *Mtb* enough to infect a normal person
- *Mtb* grow, on and off, over many years
- The cough of TB propels *Mtb* into the air
- New infections result from inhalations of infected residual of droplets

Pathogenesis of TB

- Primary TB
  - Healthy Host
  - Infection contained in the lung and/or lymph nodes
  - Progressive Pulmonary Disease
  - Extrapulmonary TB

- Latent TB infection, LTBI
  - 90% normal well
  - 10% reactivates

- Factors
  - Hematologic malignancy
  - Steroids
  - Anti-TNF
  - Aging

- Reactivation or Post-Primary TB
  - Immunocompetent or sicker
  - Pulmonary in 85%
  - Extrapulmonary in 15%
**Tuberculosis: global epidemiology**

- **Stop TB Department**
- **WHO Geneva**

**The burden of TB in 2006**

- **1.7 million deaths in 2004**
- **98% of these in developing world**
- **250,000 deaths due to TB/HIV**
- **MDR-TB present in 102 of 109 countries and settings surveyed**

**TB is the biggest cause of death from a curable or preventable infectious disease**

- **HIV/AIDS**
- **Tuberculosis**
- **Malaria**
- **Measles**

**Highest incidence rates per capita in Africa...**

- **Asia: 55%**
- **Africa: 29%**
- **Americas: 4%**
- **Eastern Mediterranean: 7%**
- **Europe: 5%**

**...but the highest number of cases is in Asia**

**...but still almost half a million cases a year in Europe, with peaks in the former USSR**
Global incidence is rising at 1%

Estimated TB incidence/100K/yr


Africa - high HIV
Africa - low HIV
World
Europe
World excl. Afr EEur

MDR-TB prevalence (%) in new cases, 1994-2003

XDR-TB – extensively and ‘extreme’ drug resistant TB

WHO recommended Stop TB Strategy to reach the 2015 MDGs

Structure of the Global Plan to Stop TB 2006-2015
Robert Koch
- developed tuberculin and injected it subcutaneously

Clemens Freiherr von Pirquet
- first use of intradermal injection of tuberculin as a diagnostic tool
- First use of the term Latent TB Infection

In Rome Omodei Zorrini was the first to demonstrate that in cattle the reactivation of TB could be prevented if the latent disease were treated with INH.

USPHS in a series of trials demonstrated the effectiveness of INH in human trials.

Testing for Latent TB Infection - LTBI

- False Negatives
  - Technical Factors
    - Application location
    - PPD improper storage
    - Reading experienced
  - Biological Factors
    - Poor nutrition or infection
    - causes e.g. HIV, measles mumps, chicken pox
    - Drug: e.g., steroids, and other immunosuppressives
    - Chronic Renal Failure
    - Malignancy
    - Stress
    - Burns, surgery
    - Age
  - Other environmental mycobacteria

- False Positives
  - Infection with Mycobacteria other than TB
    - BCG
    - M. avium complex

False Negative /False Positive TST
Species specificity of ESAT-6 and CFP-10

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Species specificity of ESAT-6 and CFP-10

Environmental strains

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<td>M xenopi</td>
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</table>

Gamma Interferon release assays

Specificity of QFT Gold

- Johnson et al,
  - 99 Low Risk subjects, Mean age 29 (18 to 59), 30% BCG vaccinated, 41% female, Specificity 98/99 = 99%
  - Clin Diag Lab Invest. 6:934-37 1999
- Mori et al,
  - 216 Healthy individuals, No identified risk factors for TB exposure, all vaccinated with BCG, Mean age 20 (18-33), 93% female, Specificity 212/216 = 98%
  - AJRCCM 170, 59-64 April 2004
- Mazurek
  - 550 with no TB risk, Only 1 positive of the low risk subjects, Specificity 449/550 = 99.8%
  - Publication in preparation

Sensitivity of QFT-Gold for active TB disease

- Mori, et al
  - 118 Culture-confirmed active TB Patients, 85% no-treatment (the rest < 7 days), Mantoux = 65.6% (5mm induration) Sensitivity 105/118 = 89%
  - AJRCCM 170, 58-64, 2004
- Ravn et al
  - 48 Culture confirmed active TB, 30 (71% - no treatment) 22 Male Age 19 to 80 years – mean 36.9 Sensitivity 41/48= 85.4%
  - Clin Diag Lab Immunol. 12, 491-6 2005
- Kang et al
  - 54 Culture -confirmed active TB Sensitivity of Mantoux 77.8 % (5mm) Sensitivity 44/54= 81.5%
  - JAMA 293, 2756-2761 2005
- Mazurek
  - 23 confirmed active TB 21/ 23 positive Sensitivity 21/23=91%
  - personal communications manuscript in preparation

US Military Recruit Reproducibility

- TST placed after first QFT-Gold test
- Subjects re-tested 4 to 5 weeks later
- Reproducibility is 99.3% after TST (n=557)

Sensitivity of QFT-Gold for active TB disease

- Mori, et al
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CDC Guidelines on the Use of QuantiFERON Gold

- "the antigens impart greater specificity than is possible with ..PPD"
- QFT-G is "as sensitive as TST in detecting infection" in untreated TB
- "QFT-G can be used in all circumstances in which TST is currently used"

TST distribution and QFT Gold results

- TST positive @ 10 mm = 314/332 (94.6%)
- QFT-GOLD positive = 33/332 (9.9%)

Results for those that developed active TB

- Contact investigation in a Japanese University
- 220 close contacts
- 242 controls (limited or no contact)
- Nearly all (>90%) BCG vaccinated
- 11 contacts identified with active TB, 1 to 6 months after exposure

Key Items 2000 ATS-CDC LTBI Treatment Recommendations

- 9 mo. INH preferred (vs 6 mo.) for HIV-
- 9 mo. INH preferred (vs 12 mo.) for those with fibrotic CXR and HIV
- 2 mo. RZ for HIV+ and HIV- under very limited circumstances
- 4 mo. Rif for HIV+ and HIV- usually when source case is INH resistant
- Under study INH 900 mg & Rifapentine 900 mg once week x 12 weeks

Baseline Medical Evaluation for Candidates for Treatment of Latent TB

- Medical history - History of TB or HIV treatment - TB exposure - Drug toxicity
- Chest x-ray - Rule out TB disease
- Laboratory data - CBC and platelets and chemistry panel, if indicated 3 sputum samples for smear, culture, and susceptibility testing if TB symptoms or findings on chest x-ray
2000 ATS-CDC Recommendations
Clinical and Chemical Monitoring

Selective use of blood tests
- Serum transaminase levels
  - HIV or HCV infection
  - Liver disease
  - Alcoholism
  - Pregnancy
- Emphasis on clinical monitoring for signs & symptoms of adverse effects (prompt evaluation and changes in Rx)
  - INH or Rm monthly
  - Rif/PZA weeks 2, 4 & 8

Clinical and Chemical Monitoring
2000 ATS-CDC Recommendations

Diagnosis of TB: Radiology

- Radiology
  - Primary TB
  - Lower Lobe Disease Dominates
  - Adenopathy Common
  - No Cavititation Unless Progressive
  - Reactivation (Post Primary)
    - Upper Lobe Disease
    - Predominates
    - Cavititation Common in Later Stages
  - Dissemination
  - Miliary
  - Local Organ Involvement

- Extrapulmonary Involvement Increases
  - Primary Disease Common
  - Atypical Presentations Predominate

- Rapid Diagnostic Tests Approved by FDA: Nucleic Acid Amplification (NAA) Tests

- Microscopy
  - 6,000 - 10,000 organisms per ml to see 3 AFB on slide
  - Acid Fast Stain
  - Auramine Fluorescent Stain
  - Repeat 3 Times
- Sensitivity
  - 55% positive on average
  - Specificity
  - 80% of CDC Verified Cases
  - 1/2-2% False Positive

- Culture
  - 100 organisms per ml to get 1 colony
  - Lowenstein Jensen Slant
  - Middlebrook 7H10
  - Liquid Medium
  - ACCUPROBE
- Sensitivity
  - 80% of CDC Verified Cases
- Specificity
  - 1-2% False Positive

- Culture
  - 100 organisms per ml to get 1 colony
  - Lowenstein Jensen Slant
  - Middlebrook 7H10
  - Liquid Medium
  - ACCUPROBE
- Sensitivity
  - 80% of CDC Verified Cases
- Specificity
  - 1-2% False Positive

- MTD
  - approved for use in AFB positive or negative samples
  - Target
    - RNA, 1000 Copies Per Bug
  - Amplification procedure
    - TMA
- Amplicor
  - approved for use in AFB positive samples only
  - Target DNA
  - Amplification PCR
Sensitivity and Specificity of MTD

<table>
<thead>
<tr>
<th>Respiratory Specimen</th>
<th>Non Respiratory Specimen</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>Catarazzo</td>
<td>Gambino</td>
<td>83.7%</td>
<td>97%</td>
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<td>Bargmann</td>
<td>Woods</td>
<td>90.6%</td>
<td>95%</td>
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<tr>
<td>Gambino</td>
<td>O’Sullivan</td>
<td>94.7%</td>
<td>100%</td>
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<td>Akasha</td>
<td>97.0%</td>
<td>99.1%</td>
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<tr>
<td>Chodera</td>
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<td>100%</td>
<td>96%</td>
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</table>

Prospective eval of 82 TB suspects
- 48 TB, 25 non TB, 9 excluded
- Sens AFB+QFT-G=96%, Spec=60%

J Clinical Micro Piersimoni Dec 2003

UCSD Experience with NAA in Pulmonary TB

Ronelle Campbell, Phung Lam, Ed Barber, Joseph Caperna, Kolpana Chalasani, Adelita Greeley, Kathleen Moser, Alyssa Tugend, Antonino Catanzaro.

- TB suspects defined as having submitted NAA on one respiratory specimen and two or more sputa for AFB

- N=60 smear + and NAA + - 58/60 (96.6%) culture positive TB
  - 2 false positives
- N=30 smear + and NAA - - 30/30 (100%) culture negative for TB
- N=15 smear - and NAA + - 11/15 (73.3%) culture positive TB
  - 4/15 false positives
- N=410 smear - and NAA - - 17/410 (4.1%) culture positive TB 13 pulm 4 extra pulm
  - 393/410 (95.8%) culture negative and TB excluded

Standard Treatment

- Induction Phase - QD 2 Months Start DOT
  - Isoniazid 300mg/dose
  - Rifampin 600mg/dose
  - Ethambutol 15-25 mg/kg/dose
  - PZA 30 mg/kg/dose
- Evaluate Sensitivity of Patient’s Isolate
- Consolidation Phase - QD to BiW for 4 Months
  - Isoniazid dose according to
    - Rifampin or Rifapentine frequency

TK Culture Medium

- Solid culture system
- Color readout
- Sensitivity = LJ
- Time to detection
  - TK - 14 days
  - JL - 28 days

Diagnosis of MDR TB

Molecular Basis of antibiotic resistance

- INH 60-70%
  - katG
  - inhA
- RM 96%
  - rpoB
- EMB
  - embCAB operon genes
    - emb-1, emb-2, emb-3
- SM
  - rpsL
- PZA
  - pncA
  - gyrA
Molecular beacon

- INH
  - Sensitivity 82.7% Specificity 100%
- RM
  - Sensitivity 97.5% Specificity 100%
  - Cal State TB Lab. Ed Desmond

What new drugs are of interest presently?

- Newer rifamycins
- Fluoroquinolones
- Oxazolidinones
- Nitroimidazopyrans
- Pyrrole derivatives
- Congeners of ethambutol
- Isocitrate lyase inhibitors
- Diarylquinolines (DARQ)
- Nitro-imidazo-oxazole derivatives
- Immunotherapies