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For Population Studies and Reproductive Health

Molecular Virology and Bioinformatics Unit

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Diagnosis and Surveillance of HIV-1 in Women and Children of South Africa

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HIV-1/AIDS in Kwazulu-Natal Why so prevalent? Why so severe?

A Wellcome Trust Programme Grant
to Study Molecular Epidemiology
and Immunopathogenesis of HIV-1 C

HIV-1 MOLECULAR VIROLOGY AND BIOINFORMATICS PROGRAM

Goals:

- comprehensive long-term virological support – MTCT intervention, ARV, vaccine and microbicide trials
- cohesive, integrated, multi-disciplinary approach
- transitional research
- practical and affordable methods for diagnosis, prevention and treatment of HIV-1AIDS

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PROGRESS TO DATE

ELISA, WB, DNA PCR, viral load, CD4, genetic subtyping, resistance genotyping, resistance phenotyping, cytokine expression (ELISA, RNA), HHV-8 serology and PCR

16s rDNA microsequencing (STDs, TB), therapeutic drug monitoring

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16s rDNA: differentiates between 1,200 strains of bacteria

- **severe and very severe childhood pneumonis**
- **TB - enormity of problem, slow growing pathogen**

Therapeutic drug monitoring

- **different subtype (macrophages, tissues)**
- **different regimens – new affordable drug combinations**
- **malnutrition and anemia,**
- **cytochrome P450**

Sequencing, Bioinformatics Courses:

Introduction: Major principles of bioinformatics – sequence databases, alignment, analysis and phylogenetics

Applied Bioinformatics: HIV-1 specialized databases, genotyping, drug resistance, genetic evolution, developing countries

Development/Maintenance of a Bioinformatics Unit: Establishing local HIV databases, Linux setup and administration, software installation, web interfaces

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Potential Impact of HIV-1 Genetic Variability on Diagnostics

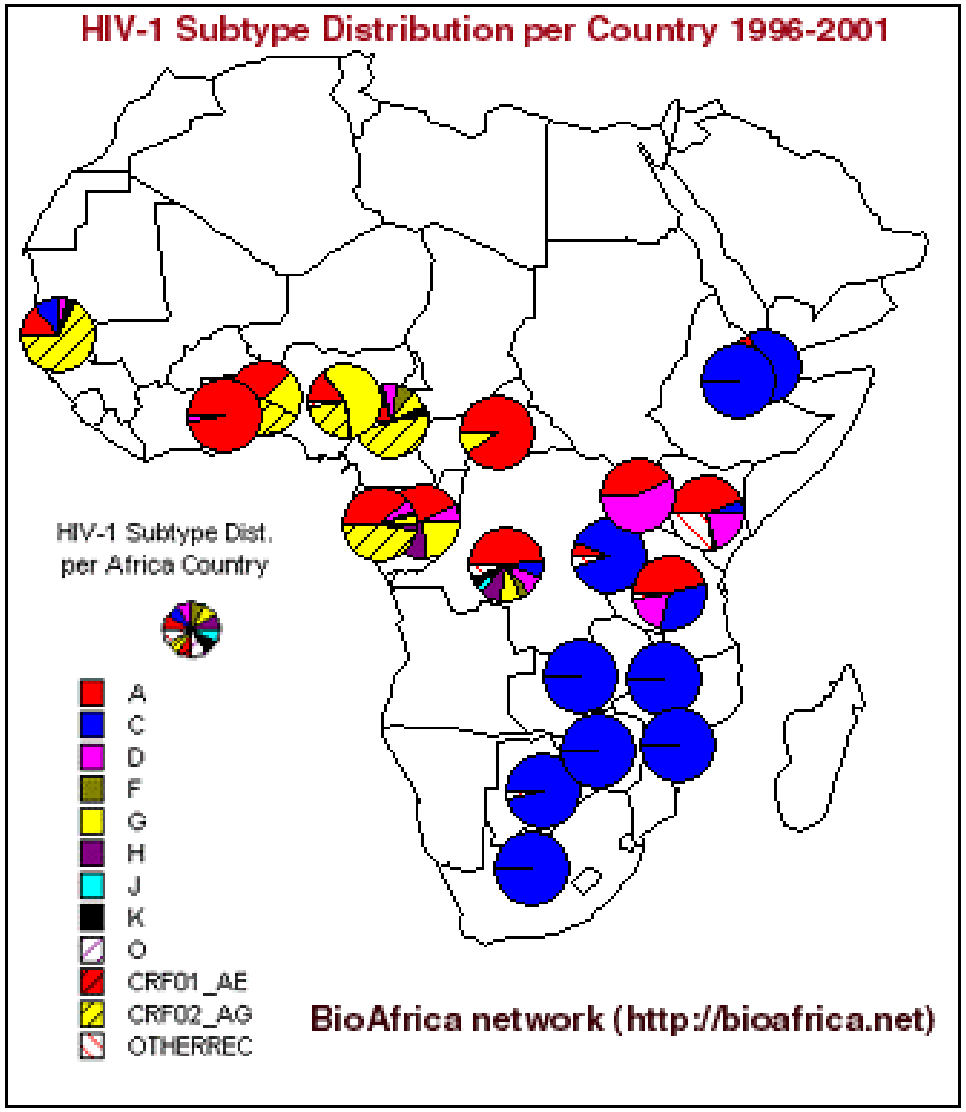
Unprecedented genetic variability
- Groups M (A to K), N, O and CRFs

Diagnostic assays developed and tested against subtype B

Includes resistance assays and interpretation of resistance

West Africa – subtype O

> 85% infections in southern Africa are subtype C



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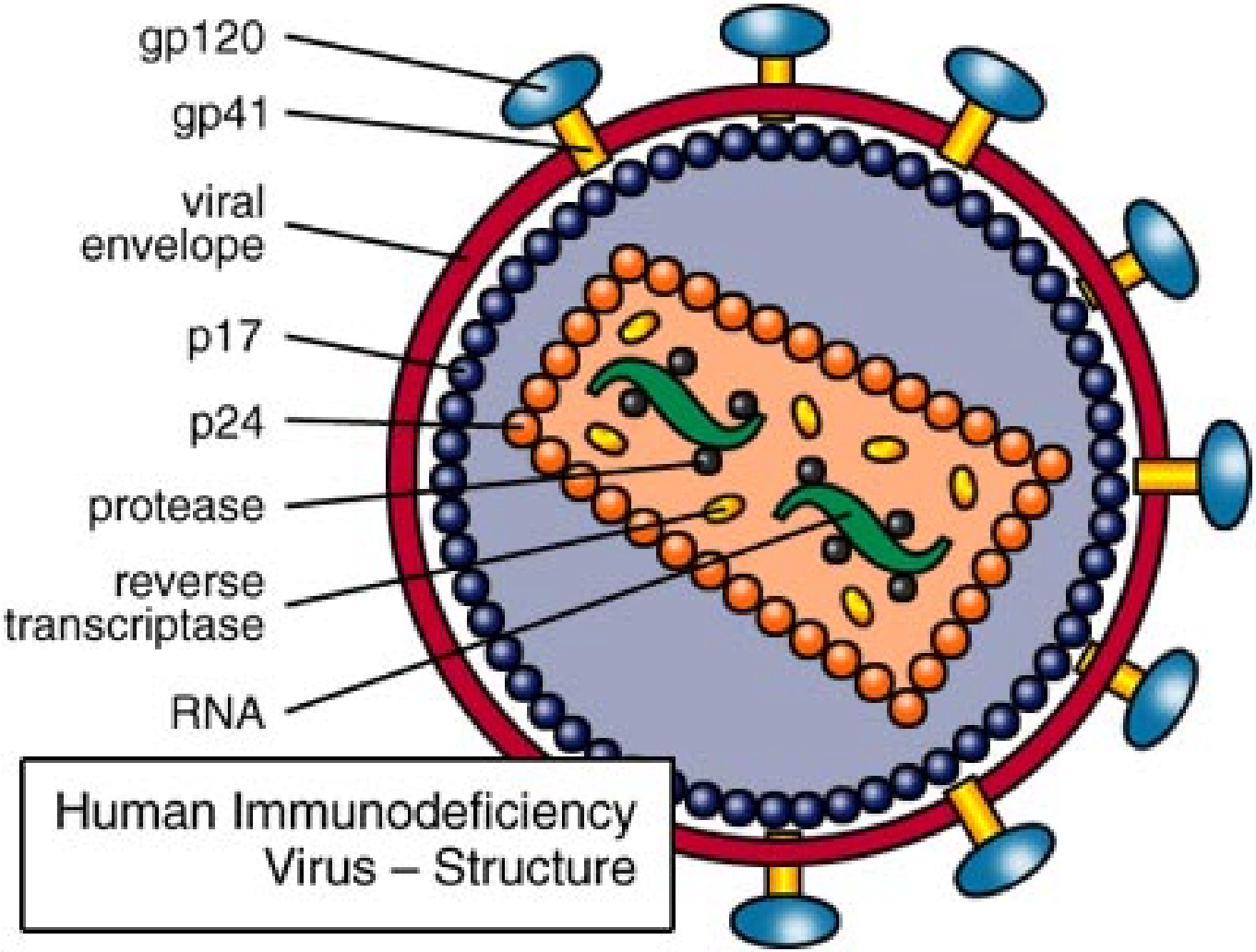

Human Immunodeficiency Virus – Structure

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Surveillance and Diagnosis of HIV-1 in Women and Children

Tests and Specimens Used for Diagnosis

Traditional

- venous blood – antibody, WB, RNA/DNA

Field Testing

- dried blood spots – antibody, WB, RNA/DNA
- Saliva - antibody

Point of Care

- rapid tests - antibody

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Surveillance and Diagnosis of HIV-1 in Women and Children

Rapid Tests: Advantages and Disadvantages

- results available same day, reduces loss to follow-up
- can be used during labour and delivery
- requires trained staff at many field sites
- difficult to provide quality assurance
- not applicable to diagnosis of infants <15 months

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Surveillance and Diagnosis of HIV-1 in Women and Children

Dried Blood Spots:Advantages

- applicable to wide range of tests - antibody, p24 atg, RNA, viral load, IgA, sequencing
- applicable to diagnosis of women AND infants
- facilitates centralization, standardization and QA
- unbiased, systematic surveillance and follow-up at population level
- small volume blood

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Surveillance and Diagnosis of HIV-1 in Women and Children

Dried Blood Spots: Disadvantages

- requires 1 to 3 days to obtain results
- not applicable to women who present in labour

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Immunization Clinic Survey of HIV-1 Infection in Rural South Africa

OBJECTIVES:

- accurate up-to-date estimates of prevalence and incidence in women and children
- accurate estimates of MTCT HIV-1 transmission rates
- by testing at 6 weeks and 9 months – better define role of perinatal (*in utero*, perinatal) vs breastfeeding

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Immunization Clinic Survey of HIV-1 in Rural South Africa

STUDY POPULATION:

- "healthy" asymptomatic mothers and children attending rural immunization clinics in Hlabisa, KZN
- all previous population-based surveillance conducted at antenatal clinics -limited to women, seroprevalence only

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Immunization Clinic Survey of HIV-1 Infection in Rural South Africa

METHODOLOGY:

HIV-1antibody and viral RNA testing of matched mother infant dried blood spots

- 988 maternal DBS
- 742 infant DBS

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Immunization Clinic Survey of HIV-1 Infection in Rural South Africa

RESULTS:

- high point prevalence – 28.2% of "healthy" women currently HIV-1-infected, microvariation
- high point incidence (1.28%) in women (antibody negative, RNA positive), annual incidence 11-16%

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Immunization Clinic Survey of HIV-1 Infection in Rural South Africa

When stratified according to age

- prevalence and incidence highest among women 21-25 years (prevalence = 38%, incidence =75%)
- women 21-25 years and teenage girls (16-20 years) accounted for large number of transmissions (45.4% for 21-25 age group; 29.1% for teenage group)

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Immunization Clinic Survey of HIV-1 Infection in Rural South Africa

Infected infants (HIV-1 RNA)

< 6 weeks	12.0%
6 wk-3 mon	31.7%
3 – 6 mon	21.1%
> 6 mon	20.3%

Lack of sensitivity – birth to 6 weeks

Decrease after 3 months illness? failure to immunize?

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Immunization Clinic Survey of HIV-1 Infection in Rural South Africa

MCTC TRANSMISSION

- overall 20.6% mothers transmitted, underestimate since 13.8% infants < 1month ; 8.2% < 2 weeks
- virus levels higher in women who transmitted (91.7% >1,600 copies/mL)

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Immunization Clinic Survey of HIV-1 Infection in Rural South Africa

CONCLUSIONS

- young women in their early twenties and late teenage years are bearing burden of AIDS epidemic in rural S. Africa
- consistent with recent results from rural Zimbabwe
- implications for targeting education and intervention efforts in rural regions, and for informed consent

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HIV-1 Component of Integrated Management of Childhood Illness (WHO/UNICEF Strategy)

Overall Objective:

- To reduce mortality in children under 5 years by improving primary health care

Specific Objective:

- To assess sensitivity and specificity of IMCI for the clinical diagnosis of symptomatic infection

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HIV-1 Component of Integrated Management of Childhood Illness (WHO/UNICEF Strategy)

Study Population:

- 690 children attending clinics in rural KwaZulu-Natal

Methods:

- HIV-1 testing of dried blood spots
  - antibodies (infants born to infected mothers)
  - RNA viral load (diagnosis, correlation with disease
- Lab-based diagnosis ve IMCI algorithm (GP) vs experienced paediatrician

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HIV-1 Component of Integrated Management of Childhood Illness (WHO/UNICEF Strategy)

OUTCOME:

- identification of children with symptomatic HIV-1
- provision of family counselling
- support and interventions needed to improve the quality of life, treatment of intercurrent infection, PCP prophylaxis
- improved nutrition

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HIV-1 Component of Integrated Management of Childhood Illness (WHO/UNICEF Strategy)

Method	No. HIV-1-infected children*
GP + IMCI	56.1%
Paediatrician	71.7%
HIV-1 RNA	100%

- 28.7% of new presentations at paediatric outclinics positive
- overall positivity 37.6% < 1 yr; 19.4% in 4-5 yr groups
- 46.6% of <15 months of age, serology not useful
- 63.6% of mothers wanted to know status of child

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HIV-1 Component of Integrated Management of Childhood Illness

Of the infections not diagnosed by IMCI practitioner

30 had either spenomegaly, hepatomegaly or parotid enlargement

- Signs not included in initial IMCI algorithm
- If included, sensitivity of diagnosis increases from 56.1% to 64.2%

HIV-1 Component of Integrated Management of Childhood Illness

Most significant clinical features:

- weight loss in mother
- generalized lymphadenopathy
- weight below 3rd centile
- weight below 60% expected for age group
- spenomegaly
- oral thrus
- parotid enlargement
- ear discharge

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HIV-1 Component of Integrated Management of Childhood Illness (WHO/UNICEF Strategy)

Conclusions:

- IMCI reasonable sensitivity, specificity
- improved algorithm simpler, more sensitive
- accepted by WHO as the new generic algorithm for countries with HIV-1 prevalence > 2%
- children with 3 or more symptoms – lab testing
- future studies in different settings

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Priorities for Laboratory Testing of Women and Infants

- expand laboratory infrastructure, consider centralized reference labs, accelerate training
- provide incentives for trainees to remain in country
- strong ongoing quality assurance programmes
- improved molecular assays that are simple, affordable, rapid and have high through-put
- develop networks to negotiate and reduce cost of testing

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Priorities for Laboratory Testing of Women and Infants

- strengthen basic and applied research capacity, mechanisms and role of breastmilk, placenta
- conduct all aspects of research including data analysis in Africa
- increased funding for applied research – development, evaluation of new assays, comparative testing
- facilitate and promote technology transfer

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