

s427 was isolated in South East Uganda in 1960¹ (blood of sheep 427) ➤ 15 passages in mice then frozen at -70°C

One 3-day passage ➤ **EATRO 217** ➤ ILRAD¹ and frozen as **ILRAD B-40** ➤ one irradiated rat for 5 days then several irradiated mice ➤ **ILRAD B-42** (16 May 1980)¹. From JJD at **ILRAD** ➤ **GAMC** ➤ one mouse for 3 days then frozen on 12 July 1983 as **RUMP 151**, which is **highly human serum resistant** so should be regarded as *Trypanosoma brucei rhodesiense* **S427**².

s427 is NOT the precursor of the ubiquitous strain we now call **Lister 427** (see below)¹.

➤ London in 1960 by KV (lost during KV move to Glasgow in 1968³)

➤ **LISTER INSTITUTE, ELSTREE** ** (from KV to BW) in 1961⁴

Shinyanga III —isolated in 1956 from cattle in Tanganyika and preserved at **EATRO** in Uganda—was also used in BW lab at the **LISTER INSTITUTE, ELSTREE** prior to and possibly concurrently with s427⁵

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LSTMH (unknown date and number of passages) frozen as⁶ **LUMP 231 & 1079**

NIMR (unknown date: could have been 2-way transfers⁴) frozen as⁶ **NIMR 15**

** 'variant 3' frozen 20 March 1964⁴ from a mouse infected from an 8-day rabbit infection by JK Miller (20 March 1962) at **LISTER**⁴. **YY** Thawed and tsetse-transmitted (two cycles) by WCG in 1992⁷ and re-frozen. PFGE pattern indistinguishable from current 'Lister 427' clones⁸. More detailed characterization published by WCG (2008) **YY** and TS (2009: a quick strain diagnostic test)⁹.

From 1961–1967 a strain designated '427' was also maintained by regular syringe passage at the London branch of the **LISTER INSTITUTE**⁴ ➤ Len Goodwin, Nuffield Institute, London, as **Lister 427** and frozen 19 April 1967⁴. ➤ **HPV** ➤ **GAMC** at **MOLTENO**

INSTITUTE in January 1970. Used for **procyclic** culture and **BF** studies prior to **VSG** work¹⁰. **PF** forms in **MCM** transferred to medium **HX1** on 12 February 1971. 5×10^6 inoculated into a mouse on 16 Feb ➤ 3 days (parasitemia 1×10^6 /ml) ➤ mouse 3 days ➤ frozen 22 February 1971 as **MI 041** ➤ cloned in mouse and frozen 24 October 1971 as **MI 060** (**VSG MITat 1.1**) ➤ **PB** lab in 1973, who probably also derived **procyclics** **YY**¹²

From BW to **RWFLeP** in 1965 or 1966⁵ as **Lister 427**
RWFLeP made clones (this is the first cloning of the lineage)
Clone 12/1c1 (1st clone from 1st peak of 12th rabbit)
➤ **HPV** ➤ **GAMC**. Frozen as **MI 042**, 19 March 1971.
➤ 1 rabbit on 12 August 1971 and prepared variant clones¹¹



MI 048 (8 September 1971) ➤ recloned 13 May 1976 ➤ **MI 121** (VSG MITat 1.6), **MI 222, 123**
MI 049 (18 September 1971) ➤ recloned 27 January 1975 ➤ **MI 099** (VSG MITat 1.5)
 18 March 1976 ➤ **MI 114, 115**
 19 March 1976 ➤ **MI 116**
 20 March 1976 ➤ **MI 117** (VSG MITat 1.4), **MI 118** (VSG MITat 1.5)
MI 052 (28 September 1971) (antigenically heterogeneous: some VSG MITat 1.6) ¥¥ Tsetse infections at ILRAD in 1993¹³.
MI 055 (4 October 1971) (VSG MITat 1.7)

MI 052 ➤ HH at ILRAD ➤ recloned in mice ➤ **FIRST CONTINUOUS CULTURE** of bloodstream-form *T. brucei*

Cultured cells recloned in mice at ILRAD, population neutralized with anti-VSG MI 052 ➤ mice and cloned the relapse population as **M4/clone2 (ILRAD Tb 033)** ➤ tsetse ➤ recloned from mouse ¥¥ (**clone 9**) ➤ **RUMP 150** (11 July 1983)¹⁴.

MI 221 (VSG MITat 1.2) cloned¹⁰ by GAMC in mice infected from ILRAD culture 5 February 1978. (➤ PB, 11 March 1980).

MI 224 (VSG MITat 1.3) cloned from relapse population in rat infected with 221¹⁵

MI 140, 141 uncloned 11-day relapse populations from two rats inoculated with 5–10 trypanosomes (22 May 1981).

April 1994, tsetse-transmitted⁷ progeny of **Lister variant 3** ➤ GAMC ➤ one mouse ➤ frozen as **RUMP 501**
 ➤ May 2004 tsetse infections¹⁶ ¥¥

April 2004, **RUMP 501** ➤ mice for 3 days ➤ culture ➤ cloned. The VSG 221 gene, which was present in the uncloned population, was lost in the clones. The expressed VSGs were cloned by RT-PCR and two different sequences were obtained from 10 clones. One clone (**501c2**) expressed a previously uncharacterized VSG and the others expressed **MITat 1.1**.

CONCLUSIONS: all extant '427' clones should be called 'Lister 427' They are almost certainly not derived from 's427'. They probably originate from the Shinyanga III strain, but this cannot be proved.

Legend The origin and peripateticism of the *Trypanosoma brucei* strain known as Lister 427

** Points between which strains could have been switched (or overgrowth of one clone from an originally mixed population) — essentially at any time between transfer of the original isolate to the Lister Institute and freezing of the ‘variant 3’ population.

¥¥ Points at which tsetse infectivity through to salivary glands has been confirmed.

VSG MITat (Molteno Institute Trypanozoon antigenic type) nomenclature is indicated for commonly used clones of Lister 427, where they have been antigenically characterized. ‘Ghost’ MITat numbers will be assigned to variants characterized elsewhere (see subsequent proposal).

Abbreviations:

INSTITUTES — EATRO, East African Trypanosomiasis Research Institute (no longer exists), Tororo, Uganda; ILRAD, International Laboratory for Research on Animal Disease, Nairobi, Kenya (now ILRI: International Livestock Research Institute); LUMP, London University Medical Protozoology, UK; MI, Molteno Institute, Cambridge, UK; NIMR, MRC National Institute for Medical Research, Mill Hill, London, UK; RU, Rockefeller University, New York, USA.

PEOPLE — BW, Bernard Weitz, Lister Institute, Elstree, UK; FO, Fred Opperdoes, Brussels, Belgium; GAMC, George A. M. Cross, New York, USA; HPV, H. Paul Voorheis, Dublin, Ireland; JDB, J. D. Barry, Glasgow, UK; JJD, Jack J. Doyle, at ILRAD; KV, Keith Vickerman, Glasgow, UK; PB, Piet Borst, Amsterdam, Netherlands; RWFLeP, Richard W.F. LePage, Cambridge, UK; SML, Sheila M. Lanham, Somerset, UK; SKM, S. K. (Dean) Moloo, at ILRAD; TS, Thomas Seebeck, Bern, Switzerland; WCG, Wendy C. Gibson, Bristol, UK.

OTHER — HX25, procyclic *T. brucei* culture medium HX25; MCM, Pittam’s monophasic procyclic culture medium (presented as an appendix to [Dixon, 1972 #4818]).

Footnotes: (Abbreviated dates are in USA format — month/day/year). Emails and letters have been compiled into Supplementary Documents.

^{2a} JDB e-mail 5/28/97

³ HPV e-mail 7/7/98

ENDNOTES (auto numbering)

¹ Original isolation described in [Cunningham, 1962 #8418]. Date of transfer from EATRO to ILRAD unknown to me. ILRAD records verified by JDB between 1978-80 (JDB e-mail 6/2/94). ILRAD B-42 was cloned by JDB (after bringing it from ILRAD according to emails 6/2/94 and 6/23/98) and tested by PB lab and shown to differ from Lister 427 in nuclear RFLPs and VSGs.

² GAMC 1983 and 1994 unpublished data.

³ KV e-mail, 2/2/95, JDB e-mails 6/2/94, 11/15/94

⁴ SML handwritten notes from a phone conversation 2 January 1973 — “...was obtained by Lister from KV in 1961 and ‘probably’ maintained by ~weekly passage in mice until 1966 when they got liquid nitrogen (but it could also have been stored in dry ice: not sure)...” — and letter . But letter from Malcolm Guy to Paul Voorheis, 17 March 1970, states that it was not frozen at Lister until 19 April 1967.

⁵ RWFLeP e-mails 6/30/98 & 7/3/98. Date of isolation of Shinyanga III based on statement in Weitz 1960 paper (R4192). But, there is a big conflict between this date and what is stated in the August 28th 1998 email from Dave Barry, which put its isolation from Glossina prior to 1950, based on records from CTVM.

⁶ These LUMP and NIMR stabilates were not available for comparison testing.

⁷ WCG letters 11/18/92 & 6/20/94 and emails.

⁸ WCG letters 11/92 & 11/93 & email 7/3/98.

⁹ Peacock L, Ferris V, Bailey M, Gibson W. Fly transmission and mating of *Trypanosoma brucei brucei* strain 427. *Mol Biochem Parasitol* 2008;160:100-6 and Kunz S, Luginbuehl E, Seebeck T. Gene conversion transfers the GAF-A domain of phosphodiesterase TbrPDEB1 to one allele of TbrPDEB2 of *Trypanosoma brucei*. *PLoS Negl Trop Dis* 2009;3:e455.

¹⁰ See [Cross, 1973 #479]. There was a period in my lab, from 1971–1976, where 427 PF and 427 BF from different sources were being grown in parallel, but it is almost certain that the PF were never re-derived from the RWFLeP clone or its progeny during this time. *T. brucei* S42 and *Trypanosoma brucei rhodesiense* ‘Wellcome strain’ were being cultured as PF at the same time. Neither of these became infectious to animals. I still have PF samples in HX25 and MCM frozen in July 1974. Although the lineage of BF and our PF 427 differs, they are undoubtedly the same. In our routine experiments, for example, we find the same patterns of VSG genes 121 and 221. Other labs (Piet Borst, Christine Clayton, perhaps) probably re-derived PF from BF. Isobel Roditi says properties and number of procyclin genes in her procyclic 427 (from Christine Clayton?) and ours differ, but I don’t know if she ever evaluated other markers to confirm if they are truly Lister 427 PF.

¹¹ For full description see [Cross, 1975 #483]. For origin and characterization of 221 see [Johnson, 1979 #4728; Hirumi, 1980 #7135; Doyle, 1980 #7137].

¹² FO letter 11/6/75 reported low Glossina infectivity (midgut only) using clone 060 fulminating infection (1 of 109 flies) but 12 of 122 flies were infected with a pleomorphic infection and metacyclic forms were seen, but no attempt made to transmit them to mice.

¹³ SKM letters of 2/21/94 & 6/27/94. A total of 532 Glossina morsitans centralis were fed on mice or goats infected with clone MI 052. Midgut and salivary gland infections were seen: from mice, 13.3% midgut and 2.3% salivary glands: from goats, 48% midgut, and 5.6% salivary glands.

¹⁴ Letter from Naseem Saigar, JJD technician at ILRAD, 7 June 1983. Would have been good to include RUMP150 in satellite DNA testing, along with Gibson clone, etc. We only checked for non-identity with 151 (s427).

¹⁵ The 224 clone was obtained by rat relapse of 221 by E. Nancy Miller at the Molteno Institute. My sample was amplified and frozen as 224A, 21 February 1978. I noted that it might be antigenically heterogeneous: 2 peaks on isoelectric focusing when I made VSG in August 1978. In sample obtained from me, the switch from 221 to 224 was characterized by loss of the 221 gene and at least 15 kb of upstream sequence and probable duplication of a large telomeric region from a > 2 Mb chromosome containing the (so-called) VSG MITat 1.3 gene [Bernards, 1986 #2064]. Sequenced by us in 2004.

¹⁶ See emails from Aksoy and Kasumba.