Stability study of *Jivantyadi Churna* used in treatment of *Tamaka Shwasa*, (Bronchial Asthma) along - With respect to baseline microbial diagnostic modalities

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ABSTRACT:

Background: Bronchial Asthma is a disease characterized by an increased responsiveness of the airways to various stimuli. It manifests by widespread narrowing of the airways causing paroxysmal dyspnoea, wheezing or cough. The drug combination selected in this study, *Jivantyadi Churna* is having properties to remove the obstruction made by *Kapha* in the *Pranavaha Srotas* and related system and normalize the functioning of *Vayu*. **Aims:** To carried out stability study of coarse powder of *Jivantyadi Churna* with respect to its stability against microbial contamination. **Materials and Methods:** Sample of prepared drug coarse powder of *Jivantyadi Churna* was prepared and studied to check microbial contamination at regular time intervals. **Results:** Every time sample was subjected to the microbiological study from the date of the preparation to the date of last microbiological study. No any contaminations were found in microbiological study. **Discussion:** Hence the present Study was carried out to observe the stability study of prepared drug coarse powder of *Jivantyadi Churna* with respect to microbial contamination of sample prepared and store in different climatic conditions and temperature. Thus a baseline microbial profile was studied at regular interval of 1 month for total 12 months (i.e. time for consumption of prepared drug). At the end of study it was found that sample was not showed presence of any microbes. **Conclusion:** In microbiological study of the prepared drug coarse powder of *Jivantyadi Churna* there were no growth found of microorganisms (bacterial or fungal), till 21th Feb 2019 i.e. 12 month from the date of preparation, shows its stability and good shelf life. Hence in present study the stability test of coarse prepared drug of *Jivantyadi Churna* with respect to microbiological findings was negative at room temperature, warm and cold, dry and humid conditions.

Keywords: Bronchial Asthma, *Jivantyadi Churna*, Microbiology Study, *Tamaka Shwasa*

Introduction:

The prevalence of Bronchial Asthma has increased continuously since the 1970s, and now affects an estimated 4 to 7% global population.[1]Asthma prevalence rates in states of India like Karnataka, Gujarat, Haryana, Uttar Pradesh and Madhya Pradesh are above the national level.[2] This disease is more predominant in children population of 6-16 years. At the age of six to seven years, The prevalence ranges from 4-32%.It is the leading cause of hospitalization for children and one of the most important chronic conditions causing elementary school absenteeism.[3,4] Geographical location, environmental, racial as well as factors related to behavior and life-style are associated with this disease.[5,6,7] Several studies had identified the prevalence of childhood asthma among Indian children.[8,9,10,11,12] As stated by W.H.O.100–150 million of global population are suffering from Bronchial Asthma, out of which 1/10th are Indians and the prevalence of asthma is increasing everywhere. Although largely avoidable, asthma tends to occur in epidemics and affects young people; asthma attacks all age groups but often starts in childhood. In India, rough estimates indicate a prevalence of between 10% -15% in 5-11 year old children.[13]

Ayurveda texts have described five types of *Shwasa Roga* and among them *Tamaka Shwasa* is one which is a "*Swatantra*" *Vyadhi* i.e. independent disease entity having its own etiological factor, patho-physiology and management. It is mentioned as *Yapya Vyadhi* i.e. a disease of chronic nature Charaka Samhita, while Sushruta considered it as *Krichchra Sadhya Vyadhi*. *Tamaka Shwasa* is basically a disorder of *Praanavaha Srotasa* while other *Srotasa* are also vitiated.

This alarming raise in the prevalence of Bronchial Asthma can be accounted to factors such as atmospheric pollution, rapid environmental changes, adaptation of newer dietetic preparations and tremendous psychological stress. It calls the attention of medical world due to significant burden in terms of health care costs as well as lost productivity and reduced participation in family life. The drug was prepared in pharmacy of Gujarat Ayurved University, Jamnagar. No any extra preservative was added to the test drug. Drug preparation was finished on 06 January 2018. Finished product was stored in airtight plastic container at room temperature. Thus in the present study on attempt was taken to check stability of coarse powder with respect to its Microbial profile at different climatic conditions and temperature setups at regular interval for a period of 12 months.

Aim:

To study the stability of finished product and to check microbial contamination in the finished product at different time interval- at different climatic conditions, temperature and humidity set ups.

Materials and Methods:

Samples of coarse powder of *Jivantyadi Churna* was prepared and studied to check microbial contamination at different climatic conditions. The study was conducted at Microbiology Laboratory, IPGT & RA, GAU, Jamnagar, Gujarat, India.

Preparation Time:

Drug was prepared under SOP with the utmost care to avoid any sort of contamination.

Date of preparation: 06.01.2018

Storage: Drugs was stored in plastic containers at room temperature in a dark and dry place. Samples were subjected to stability study with respect to microbial and fungal contamination at regular intervals. Details of which are cited below.

Microbial profile: Microbial contamination was assessed by two methods to check any mycological findings and bacteriological findings.

1 **Smear Examination-**

Wet mount /10% K.O.H. Preparation

Gram's stain

2 **Culture Study-**

Fungal culture

Aerobic culture

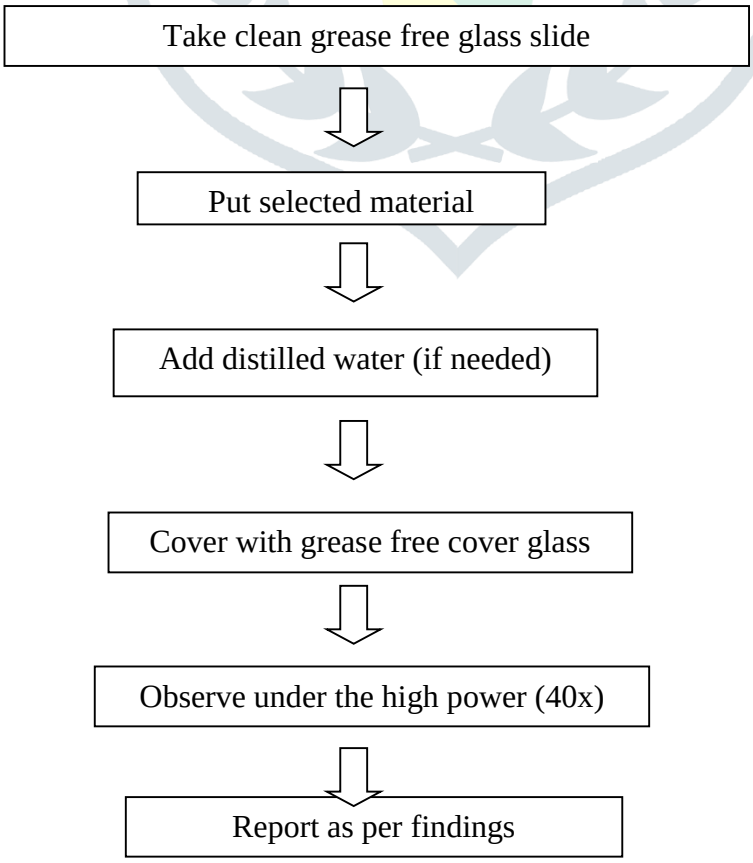
The details of the procedures followed are given below.

1. Smear Examination:

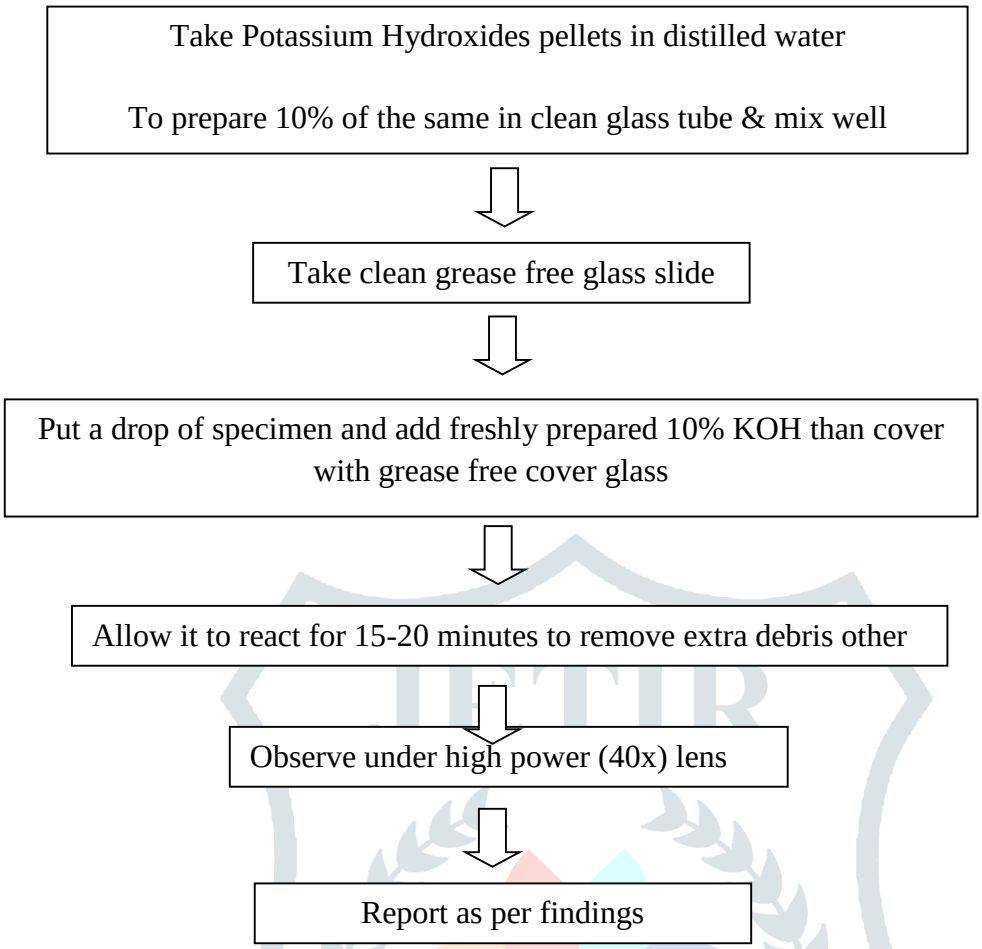
A. Wet mount /10% K.O.H. Preparation:

Aim: To rule out any mycological findings.

Specimen: ***Jivantyadi Churna***



Procedure For 10% KOH preparation



B. Gram's stain test:

Gram staining is a differential staining technique that differentiates bacteria into two groups: gram-positive and gram-negative. The procedure is based on the ability of microorganisms to retain color of the stains used during the gram stain procedure. Gram-negative bacteria are decolorized by any organic solvent (acetone or Gram's decolorizer) while Gram-positive bacteria are not decolorized as primary dye retained by the cell and bacteria will remain as purple. After decolorization step, a counter stain effect found on Gram negative bacteria and bacteria will remain pink. The Gram stain procedure enables bacteria to retain color of the stains, based on the differences in the chemical and physical properties of the cell wall (Alfred E Brown, 2001)[14]

Aim: To rule out any bacteriological findings.

Specimen: ***Jivantyadi Churna***[15]

Procedure for Gram's stain

Take clean grease free glass slide to prepare dry equal thick Preparation (i.e. smear)

⇩

Fixed prepared smear by passing 3-4 times over the flame of Bunsen burner (The fixation kills vegetative form of microbes and render them permeable to stain, make material stick to the surface of slide & prevent autolytic changes)

⇩

Cover fixed prepared smear with **Gram's crystal violet** solution and allow to remain for mentioned time as per kit procedure

⇩

Washed off smear to remove excessive reagent with tap water

⇩

Cover smear with **Gram's Iodine** solution and allow remaining for mentioned time as per kit procedure

⇩

Washed off smear to remove excessive reagent with tap water

⇩

Decolorize smear with **Gram's decolourizer** by holding the slide at slope position and pour gram's decolourizer – acetone from its upper end up to removal of colour of primary dye (i.e. Gram's Crystal Violet) or as per kit procedure

⇩

Washed off smear to remove excess acetone with tap water

⇩

Cover smear with **Safranin** solution and allow to remain for mentioned time as per kit procedure

⇩

Washed off smear to remove excessive reagent with tap water

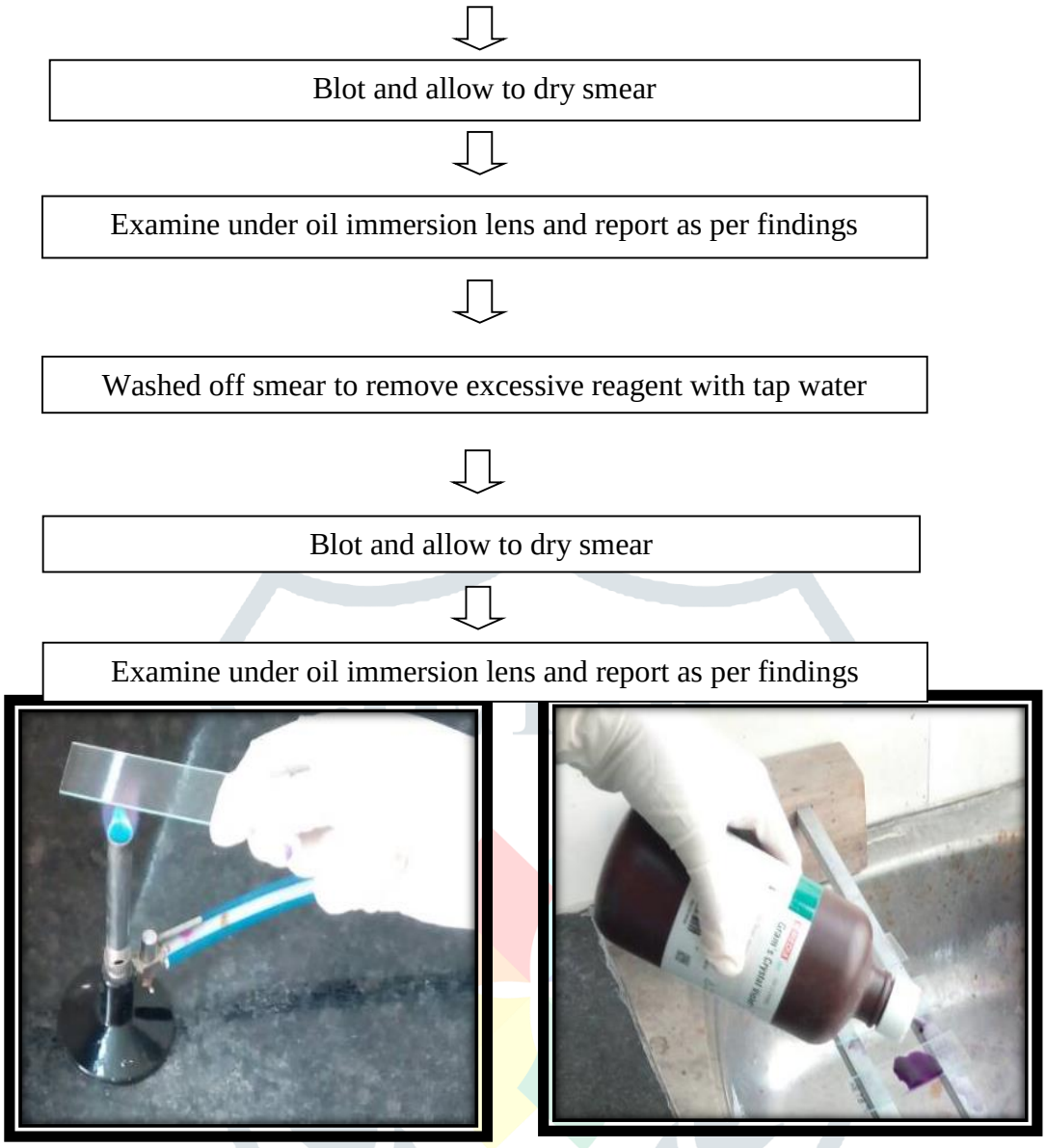


Figure 1. & 2. Smear staining Procedure

1. Culture Study

A. Fungal culture method:

Respected materials collected with sterile cotton swab for inoculation purpose on selected fungal culture media (i.e. an artificial preparation).

Name of media : Sabouraud Dextrose Agar Base (SDA),
Modified (Dextrose Agar Base, Emmons)

Company : HIMEDIA Laboratories Pvt. Ltd.

Required time duration : 05 to 07 days

Required temperature : 37 ºC

Use of media : For selective cultivation of pathogenic fungi.

Figure 3. Sabouraud Dextrose Agar Base (SDA) bottle

Procedure for fungal culture

In the clinical microbiology laboratory culture method are employed for isolation of organisms (The lawn / streak culture method is routinely employed)

⇩

Choose appropriate selective solid media for inoculation purpose

⇩

Dry selective solid media in Hot Air Oven before specimen inoculation Allow cooling dried medium before Specimen inoculation

⇩

Inoculate selective specimen by Sterile cotton swab or by Nichrome wire (24 S.W.G. size) loop [First sterile loop in Bunsen burner oxidase flame-blue flame and allow it cool than loop is charged with selected specimen to be cultured. One loopful of the specimen is transferred onto the onto the surface of well dried

⇩

After inoculation / streaking process incubate inoculated medium in inverted position at 37^0 c for 05 to 07 to 21 days in incubator (incubation days are as per growth requirement) under aerobic atmosphere

⇩

After selected incubation period examined growth by necked eye in form of colony or arial growth and confirm growth by performing different related biochemical reactions and different related staining procedures. After that report isolates.

B. **Aerobic culture method:**

Respected materials collected with sterile cotton swab for Inoculation purpose on selected aerobic culture media (i.e an artificial preparation).

Name of media: MacConkey Agar (MA) and Columbia Bloodagar (BA)

Company : HIMEDIA Laboratories Pvt. Ltd.

Required time duration : 24 to 48 hours

Required temperature : 37 ºC

Use of media : For selective cultivation of pathogenic bacteria.

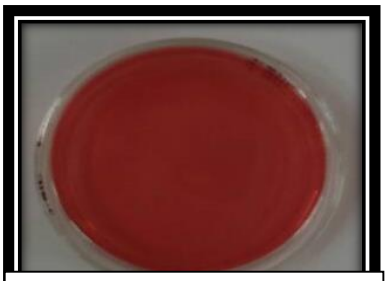


Figure 4. MacConkey Agar (MA)

Procedure for Aerobic Culture

In the clinical microbiology laboratory culture method are employed for isolation of organism (The streak culture method is routinely employed)

⇩

Choose appropriate selective solid media for inoculation purpose

⇩

Dry selective solid media in Hot Air Oven before specimen inoculation, Allow to cool dried medium before specimen inoculation

⇩

Inoculate selected specimen by four flame method (the loop should be flamed and cooled between the different sets of streaks i.e. four time) on surface of cool dried medium with nichrome wire (24 S.W.G. size) loop [first sterile loop in Bunsen burner oxidase flame –blue flame and allow it to cool than loop is charged with selected specimen to be cultured. One lapful of the specimen is transferred onto the surface of well dried plate]

⇩

After streaking process incubate inoculated medium in inverted position at 37^0c for 18-24 hours in incubator under aerobic or 10% CO_2 atmosphere

⇩

After selected incubation period examined growth by nacked eye in form of colony and confirm growth by performing different related biochemical reactions and different related staining procedures.

After that report isolates

OBSERVATIONS AND RESULTS:

Every time samples were subjected to the microbiological study to rule out stability of prepared drug up to consumption of the same. Results are shown in table no 1.

Table 1: Showing observations of sample *(Jivantyadi Churna)* preserved at room temperature

Sr. No	Days of investigations After preparation of the sample	Date of Sample given	Storage at….		Observations of sample (*Jivantyadi Churna*)			
			Humidity	Temp. (^{0}C)	Gram's Stain	Aerobic culture	Wet mount/ 10% KOH preparation	Fungal culture
1.	65 Days	12/03/18	44%	29^{0}C	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	95 Days	12/04/18	30%	34^{0}C	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3.	128 Days	14/05/18	30%	37^{0}C	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	149Days	04/06/18	48%	35^{0}C	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
5.	185 Days	06/07/18	54%	33^{0}C	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6.	219 Days	13/08/18	78%	28^{0}C	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
7.	247 Days	10/09/18	73%	28^{0}C	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
8.	277 days	10/10/18	37%	36^{0}C	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

9	311 days	13/11/18	52%	29^0C	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
10	341 days	13/12/18	31%	24^0C	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
11	375 days	16/01/19	35%	24^0C	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
12	411 days	21/02/19	56%	26^0C	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

Discussion:

For better safety and efficacy, drug should be free from any type of microbial contamination. Stability of drug is expressed in term of its Shelf life. The factors affecting stability of prepared drug are categorized under intrinsic and extrinsic factor (FDA report 2001). Intrinsic factors include moisture content, acidity, nutrient content, biological structure, redox potential, naturally occurring and added antimicrobials. Extrinsic factors include types of packaging, effect of time/temperature on microbial growth, storage/holding conditions and processing steps (FDA report 2001). Microbial contamination should avoid increasing drug stability and storage time. Coarse powder of *Jivantyadi Churna* was prepared and stored at room temperature. Sample was selected randomly for study of microbiological contamination. Changes in temperature and humidity of environment were observed during study period. Optimum temperature for microbial growth is temperature at which microbes multiplies, this optimum temperature for psychrophilic bacteria (cold loving bacteria) is 15-20 ^{0}C while for mesophilic bacteria (middle loving) and thermophilic (heat loving) bacteria is 30-37 ^{0}C and 50-60 ^{0}C respectively. The region where the drug was prepared and sample was stored was very proximal to sea coast, this area has longest sea shore and maximum number of sea ports, so relative humidity (RH) remains high in all the seasons of the year[16]. High RH may allow the growth of microbes, RH remain variable during whole study period, although air cannot be considered dry at RH more than 40%. Wet mount, fungal culture, gram stain and aerobic culture tests were used to study fungal and bacterial contamination in the sample of monthly interval from 10th Feb 2018 to 13th Feb 2019. During this study period no any microbes were isolated as a result of aerobic culture and no any fungal pathogen were isolated as a result of fungal culture (as shown in Table 1). Moisture content of drug play important role in its long term storage. Moisture contents main causative factor in drug deterioration, it also acts as an enzymatic activator which slowly decomposes the drug resulting in its degradation.

Probable reason for no contamination along with good shelf life. As *Katu* and *Tikta Rasa* is said to be *Krimighna* and absorb the excessive moisture. Drugs like *Ela*, *Tulsi*, *Bharangi*, *Aguru*, *Pippalimool*, *Nagkeshara* and *Twaka* itself having long shelf life properties. In addition manufacturing, packing and storage of both the drugs have done with SOP guidelines with precautions and care. These were also the

important contributory factors for this success in prevention of microbial contamination and good shelf life of drugs.

Conclusion:

Shelf- life is the time period from when the product is produced until the time it is planned to be consumed or used. Several factors are used to determine a product's shelf-life, ranging from organoleptic qualities to microbiological safety. Hence microbiological study of coarse powder of *Jivantyadi Churna* showed that the quality of *Churna* was found in standard condition. There were no growth found of microorganisms (bacterial or fungal), till 21st February 2019 i.e. 12 months from the date of preparation, shows its good shelf life.

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